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Student Login. Room Name. JOIN. English. **Socrative Student Response by MasteryConnect.**

A screenshot of a web browser window showing the Socrative Student login interface. The browser's address bar displays the URL <https://b.socrative.com/student/>. The main content area features the Socrative logo with the tagline "by MasteryConnect". Below the logo is a "Student Login" form. The "Room Name" field contains the text "CHOTTEAU". A large orange "JOIN" button is positioned below the room name input. At the bottom of the page, there is a language selection dropdown set to "English" with the United States flag icon. The footer of the page reads "Socrative Student Response by MasteryConnect".

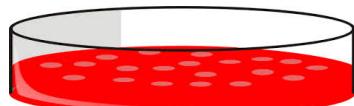


Biopharmaceutical production process development and manufacturing

Veronique Chotteau, KTH (Royal Institute of Technology), Stockholm, Sweden

- *Cell Technology Group, Dept. Industrial Biotechnology, CBH School*
- **AdBIOPRO** *AdBIOPRO, Competence Centre for Advanced Bioproduction by Continuous Processing*
-  *iConsensus, EU project - Innovative Medicines Initiative*
-  **WCPR**
Wallenberg Center for Protein Research *Wallenberg Centre for Protein Research – MedImmune AstraZeneca*
- *Centre for Advanced Medical Products*

Process



Cultivation



Purification

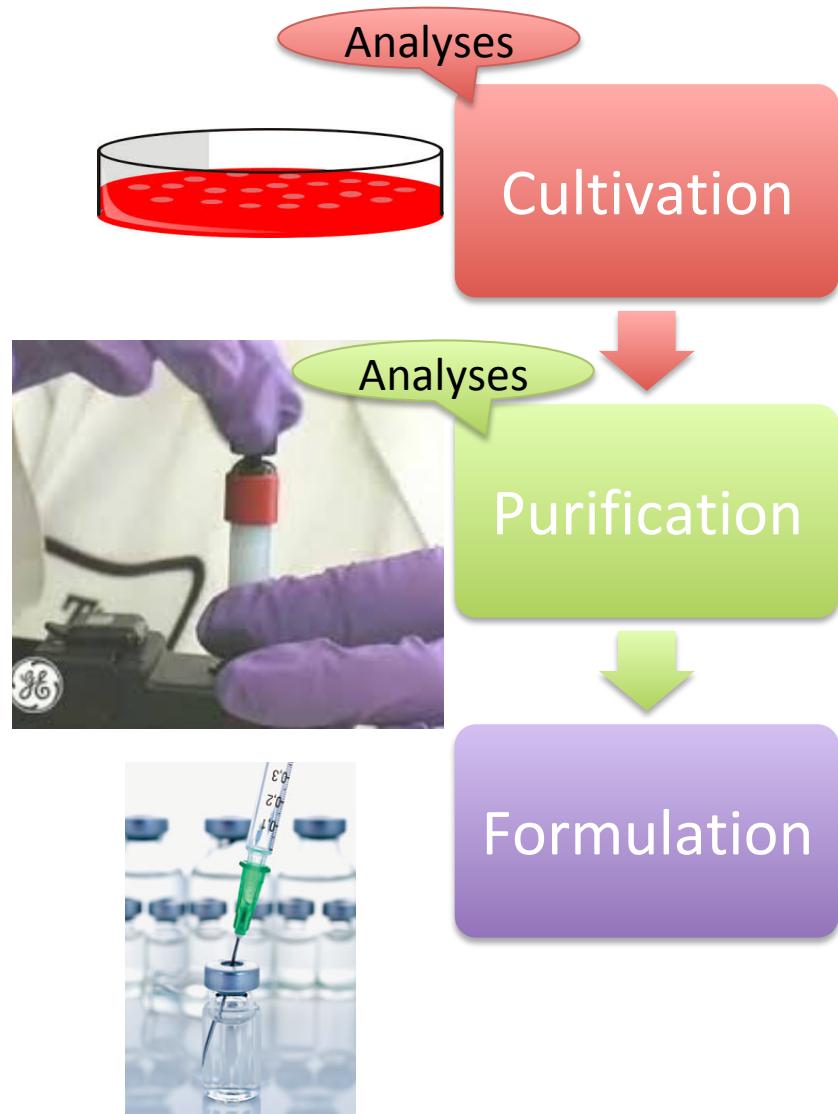


Formulation



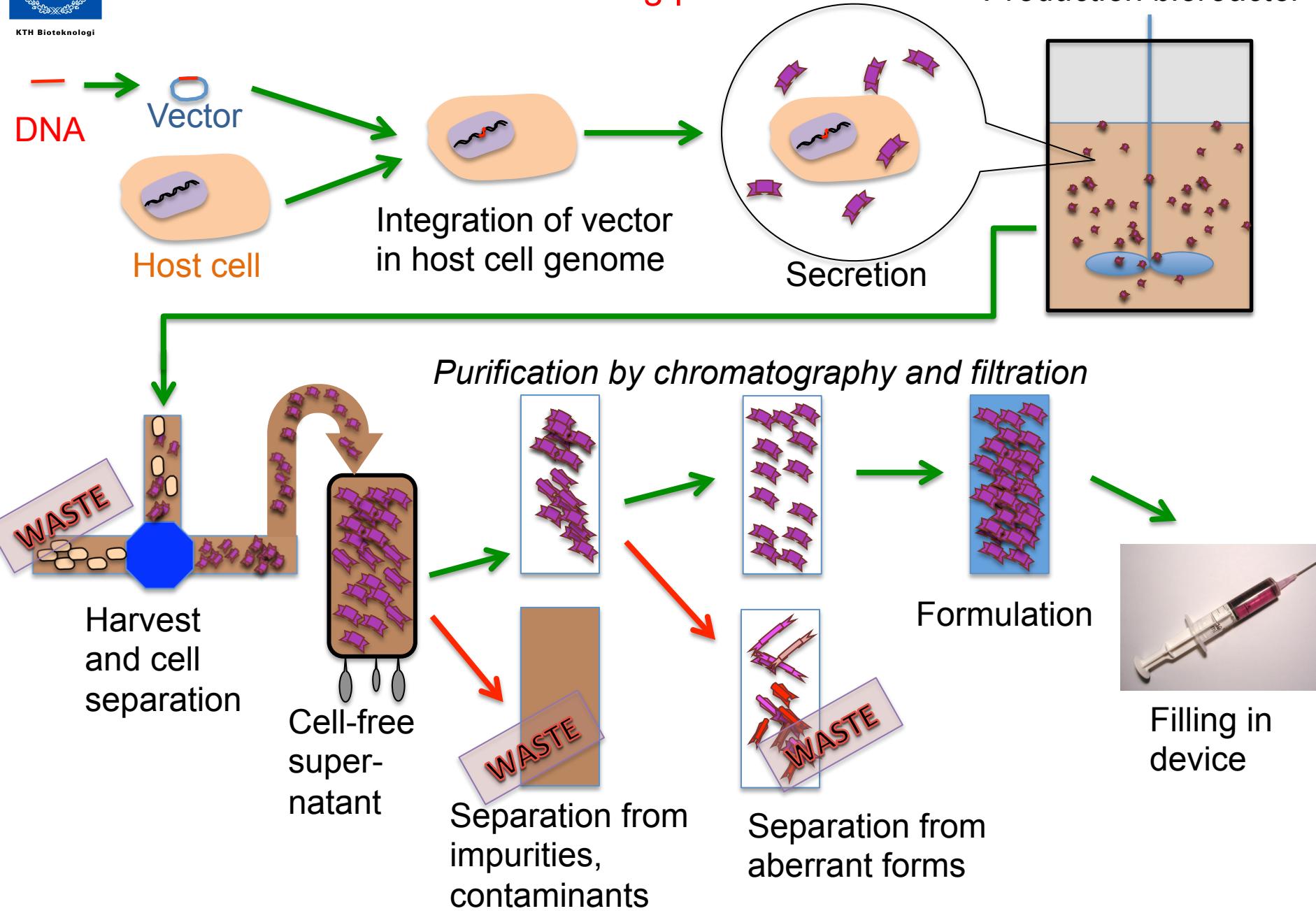
source: Bioengineering, GEN EngNews, GE Healthcare

Process

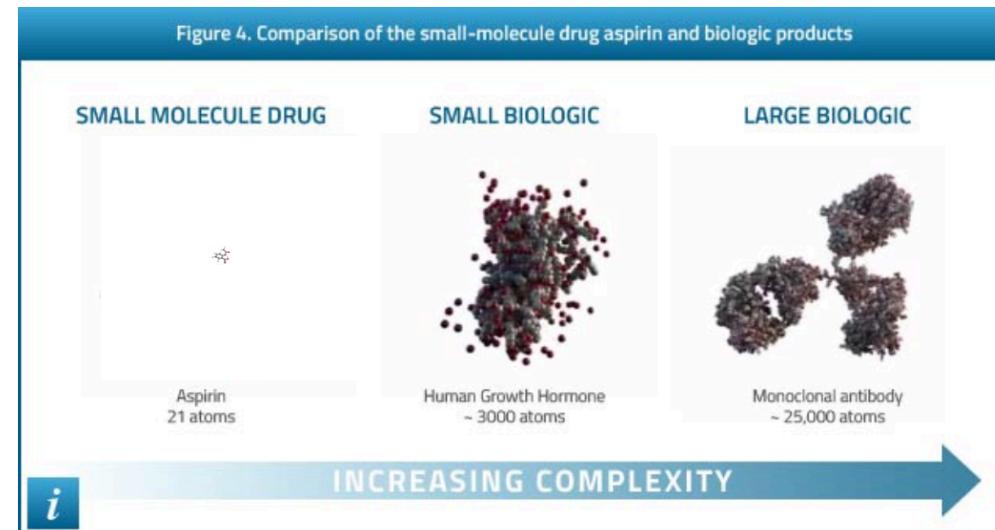


source: Bioengineering, GEN EngNews, GE Healthcare

Animal cell-based manufacturing process

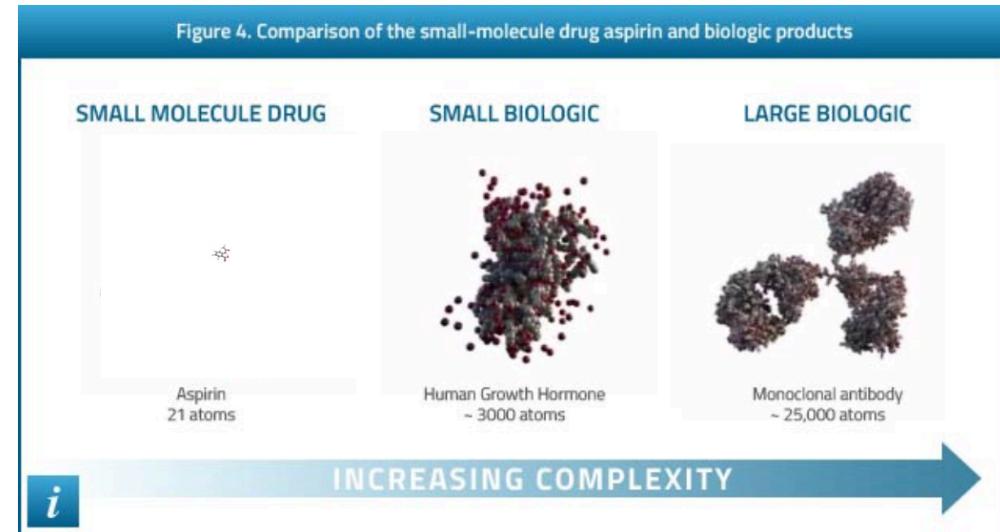


Biopharmaceutical production versus small molecule drug production



What are the main differences between biopharmaceutical production versus small molecule drug production?

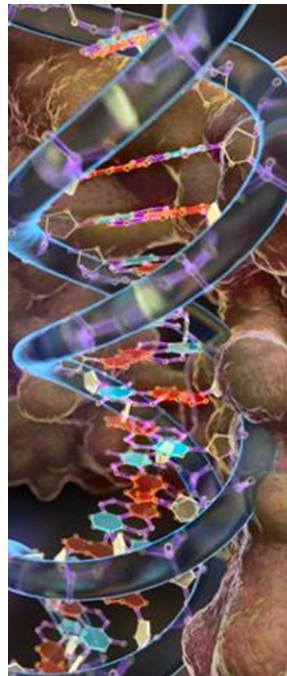
Biopharmaceutical production versus small molecule drug production



	Small molecule drugs	Biopharmaceuticals
Molecular weight	200 to 600 g/mol	20000 to 800000 g/mol
Cost	\$	\$\$\$
Production	Organic synthesis with single molecule as outcome	Produced by living cells with varying molecule as outcome
Purity	Absence of toxic by-product	98-99% since injected in blood stream
Safety	No risk of viral contamination	More stringent since cells can carry viruses, etc.
Process	Different processes can produce the same drug	The process is part of the biopharmaceutical identity Each process is unique 7

Purpose of process development

- Development of a process able
 - to be produced in pilot scale for injection in human → **clinical phase I/II**
 - enough quantity
 - ensured quality of biopharmaceutical
 - correct biological function and quality attributes such as glycosylation, absence of truncation/cleavage, absence of aggregation, correct subunit association, di-S bridges, phosphorylation
 - correct purity, i.e. absence of impurities introduced by the process and aberrant forms of the biopharmaceutical
 - patient safety
 - to be potentially scaled-up to commercial size, e.g. no change of cell line, acceptable cost of goods → **yield**
 - **clinical phase III** → 'commercial process' → only minor changes and process characterisation
- As fast as possible and as cheap as possible



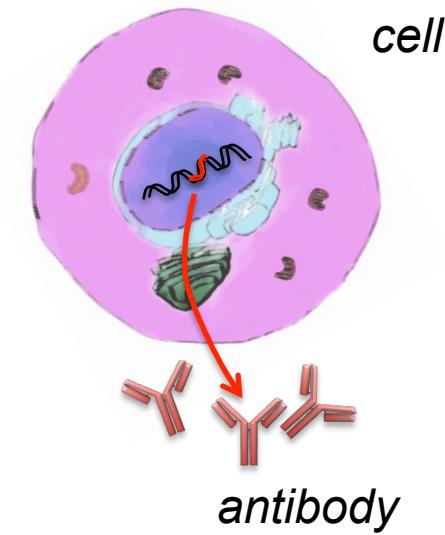
Cell system and Cultivation

source: A Racher, Lonza (2015)





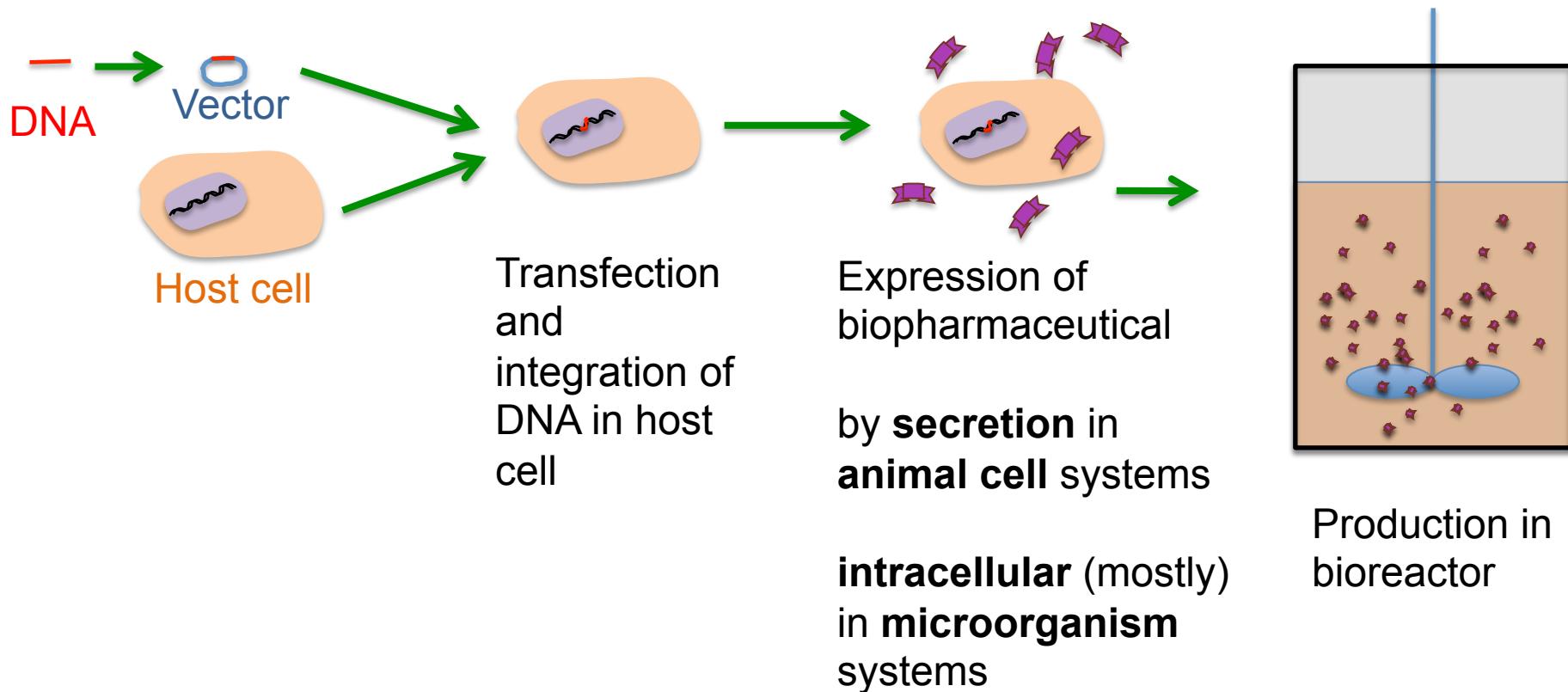
Cell systems



From candidate drug to commercial product

Biopharmaceutical molecule candidate <-> gene (**DNA**) coding for this molecule

gene → integrated in a host **cell** → production of biopharmaceutical





Which host cells are used to produce the biopharmaceutical of your project?

Host cells for biopharmaceuticals

Examples

- Insulin - diabetes

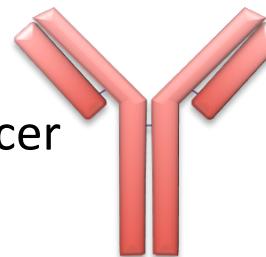


- Growth hormone

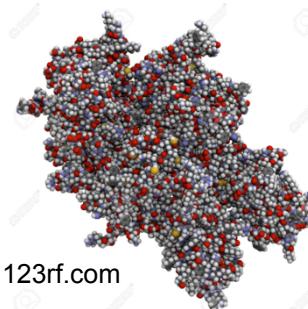


- Antibody

- Herceptin - Breast cancer
- Humira – Arthritis

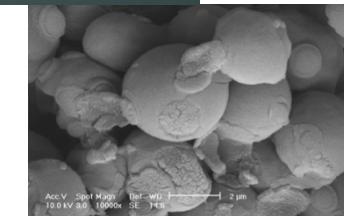
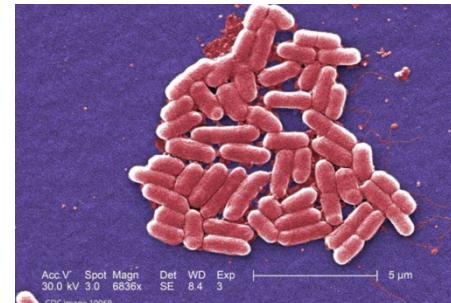


- Coagulation Factor VIII - hemophilia



source 123rf.com

source pritzkerlaw.com



source Ekpeni et al 2014

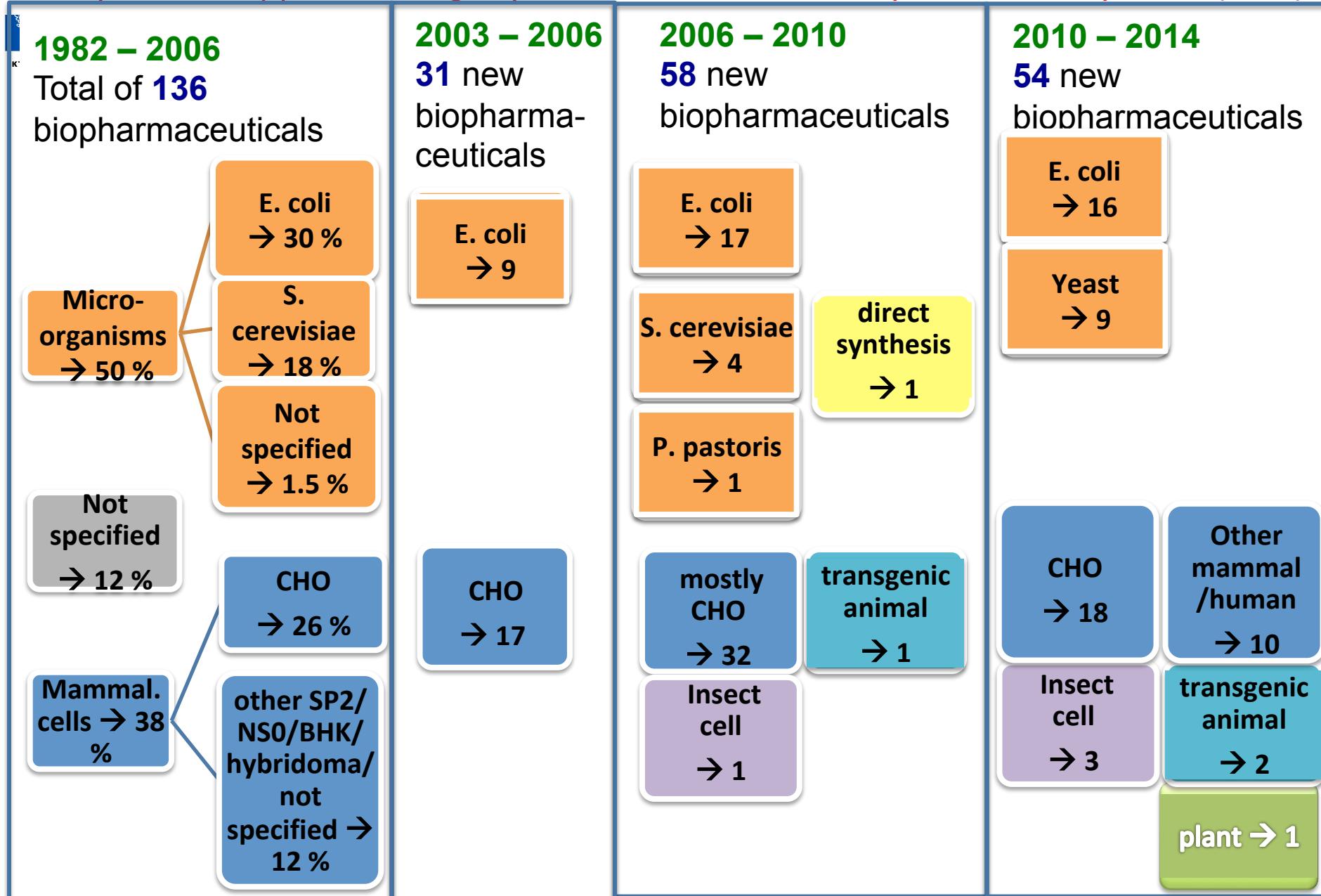
Produced by bacteria or yeast

Produced by mammalian/human cells

source Anna Lindqvist, CETEG, KTH



Cell systems – Approved drugs by FDA & EMA → tendency = mammal systems (CHO)

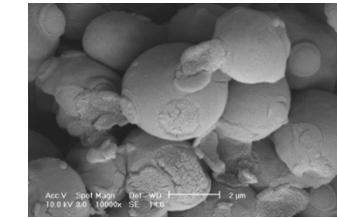
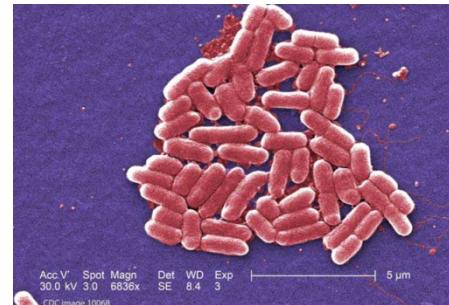


Source: G. Walsh 2006, G. Walsh 2010, G. Walsh 2014

Microorganism systems for the production of biopharmaceuticals

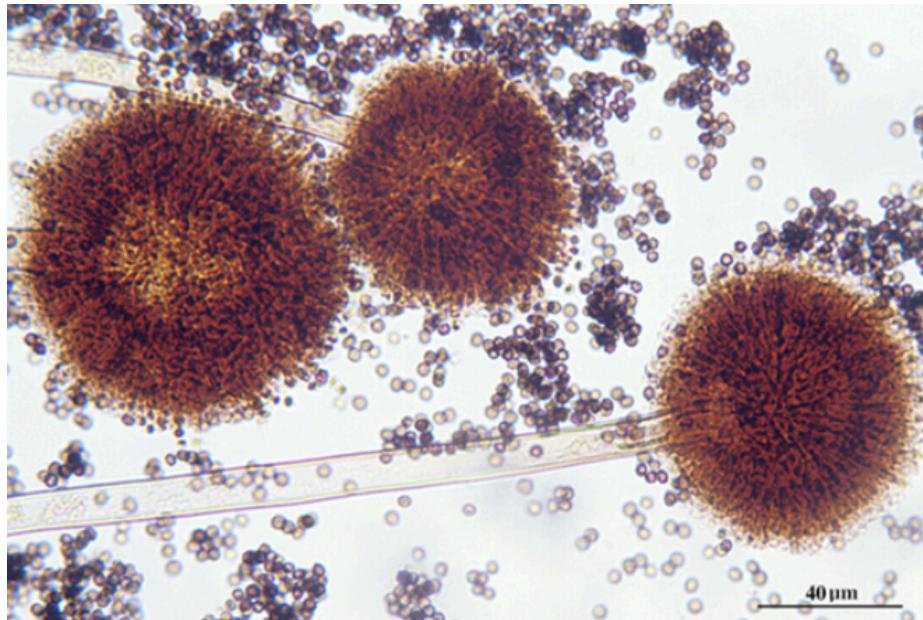
- Bacteria: *Escherichia coli* (*E. coli*)
- Yeast: *Saccharomyces cerevisiae*
- Yeast: *Pichia pastoris* (e.g. vaccine against hepatitis B; human serum albumin)
- Filamentous fungi: *Aspergillus niger* (ex: DSM, Talactoferrin clin. phase 2),

source pritzkerlaw.com



source Ekpeni et al 2014

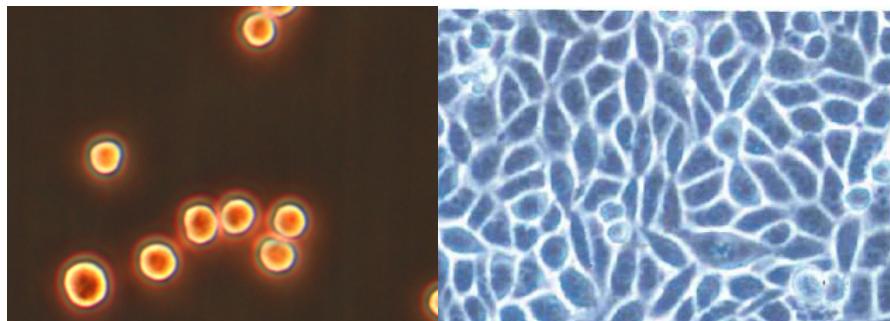
source MBBS medicine



Animal cell systems for the manufacturing of biopharmaceuticals

Chinese Hamster Ovary (CHO) → derived in 1950 in Puck's lab from Chinese hamster ovary, tumoral, extensively used for research, a lot of mutant variant exist. Variant important for biopharmaceutical industry developed in Chasin's lab deficient in dehydrofolate reductase.

Exist as suspension or adherent cells



Anna Lindqvist, CETEG, KTH

Source: Freshney, 2005

Hybridomas → production of monoclonal antibodies, B-cells from the spleen of an animal fused with myeloma tumor cells (invented by Cesar Milstein, Georges J. F. Köhler and Niels Kaj Jerne in 1975) today for research & diagnostics

OBSOLETE for manufacturing

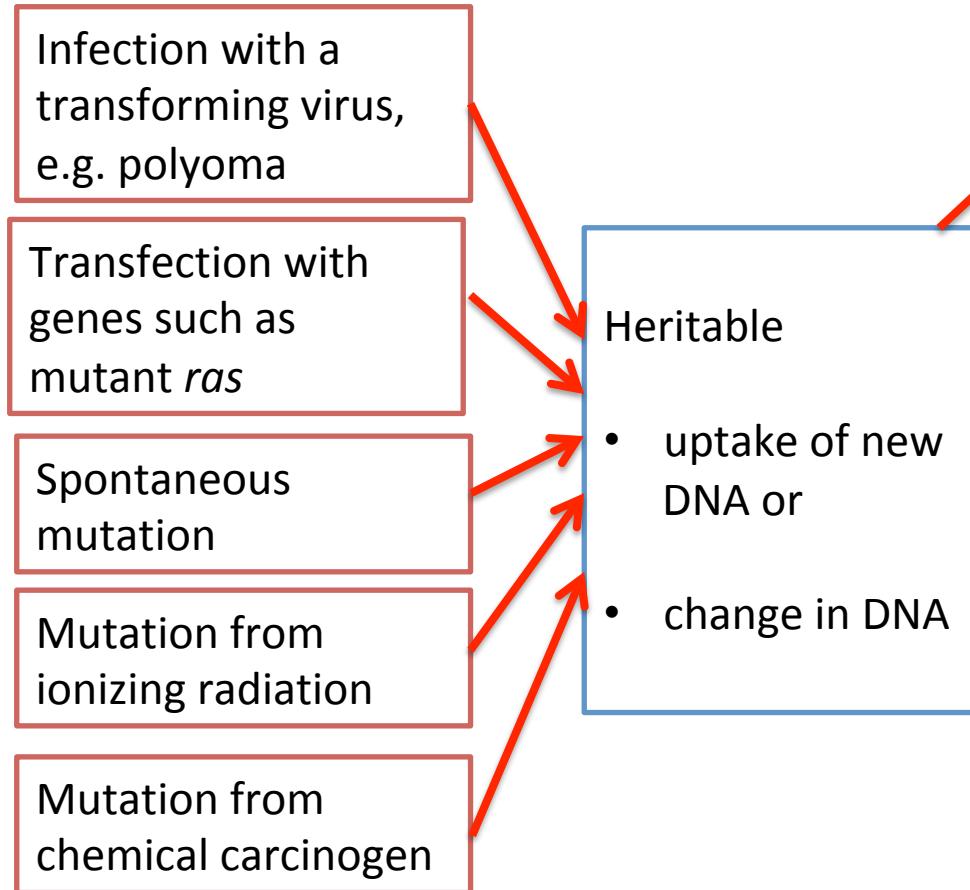


Cell systems for the production of biopharmaceuticals (cont')

- Mouse myeloma cells NS0, SP2/0, murine lymphoid both derived from BALB/c mouse, parental cell = differentiated B cell, inherently capable of producing high level of immunoglobulin, but lacking the ability to produce IgG
- Baby Hamster Kidney (BHK) isolated by Stoker and McPherson from an unusually rapidly growing primary culture of new born hamster kidney tissue
- Human Embryonic Kidney 293 cells (HEK293) (Van der Eb & Graham 1977) transformation of healthy cells by adenovirus.
- Per.C6 (human retina) immortalized by transformation with adenovirus 5 DNA
- Baculovirus in insect cells: virus-like particles vaccine
- Vero cells (African Green Monkey Kidney)
Yasumura & Kawakita 1962 → *production of virus*
- Avian (e.g. EB66 derived from duck embryonic cells, Vivalis) →
production of virus (influenza) or biopharmaceuticals

Established mammalian/human cell lines are immortal

Transformation of mammalian cells to become immortal



Change in phenotype, e.g.

- growth rate,
- metabolism,
- anchorage dependency,
- longevity,
- tumorigenicity,
- differentiation,
- contact inhibition

Transformed and stem cells often express the enzyme telomerase

→ *replication of DNA terminal sequence*

→ *life span extension*

→ *infinite growth*

Comparison of the main systems

	E. coli	Yeast	Mammal cells
Drug size	small < 50 kDa	small & large	small & large
Post-translational modifications (e.g. folding, cleavage, subunit association, glycosylation, phosphorylation, carboxylation)	no glycosylation (or very limited)	different glycosylation (risk for immunogenic reaction)	glycosylation similar or close to human
Conformation/folding	correct / need of refolding / uncorrect	correct	correct
COGS (Cost of Goods Sold)	low	low	high
Duration of development	shorter	shorter	same or longer
Duration of production (clinical phase and manufacturing)	shorter	shorter	longer
Patient safety	good	good	good

Glycosylation in CHO and NS0 systems and non mammal systems

- CHO – Chinese Hamster Ovary cells
 - (in principle) no problem
- NS0 – murine myeloma
 - can carry immunogenic sugar groups (potentially high mannose, alternative sialylation, galactose in position alpha(1-3) instead of beta(1-4))
- yeast
 - immunogenic sugar groups (high mannose)
- plant
 - immunogenic sugar groups (high mannose, xyl beta(1-3), fuc alpha(1-3))
- insect
 - immunogenic sugar groups (high mannose)

Classical strategy for the choice of production system

? molecule -> glycosylated

? glycosylation important for biological activity or for half life

>> YES → mammal cells (or transgenic mammal)

>> NO

→ ? molecule size < 50 kDa >> YES → E. coli

→ ? very large molecule >> YES → try yeast and mammal cells (or insect cells, plants, fungi)

→ medium size → try E. coli also

→ Rm: even sometimes ‘small’ molecules are too ‘difficult’ for E. coli

Production level in bioreactor

Production level in g / liter of culture

→ highly protected information (!)

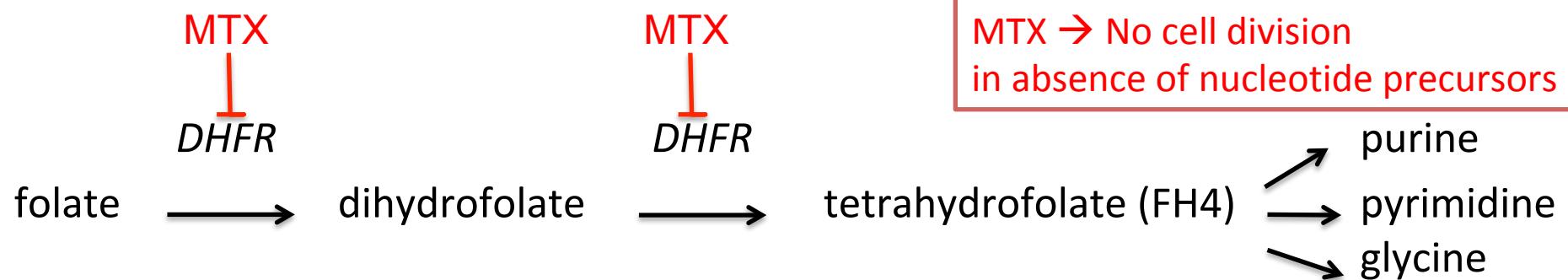
Numbers below are approximations

- hybridomas → 0.1 – 1
- SP2 and NS0 → 0.5 – 5
- CHO → 0.05 – 5 → 10 → large potential and knowledge
- BHK → 0.1 ?
- HEK293 → 0.1 - 2
- Per.C6 → 0.3 → 10

- E. coli → 1 – 4 → 15
- Yeast(*S. cerevisiae*) → 3
- Yeast(*Pichia pastoris*) → 5
- Fungi → ≈ 1

Cell expression for mammalian cell lines – industrial systems

DHFR – Dihydrofolate reductase



Methotrexate (MTX) = analog to folate → binds to DHFR and inhibit FH4 production

- Cells are overproducing DHFR in presence of MTX to survive and divide
- Transfection of gene of interest and DHFR gene together in DHFR⁻ cells
- Exposure to increasing concentrations of MTX → the cells do more and more copies of DHFR and of the gene of interest

GS – Glutamine synthetase



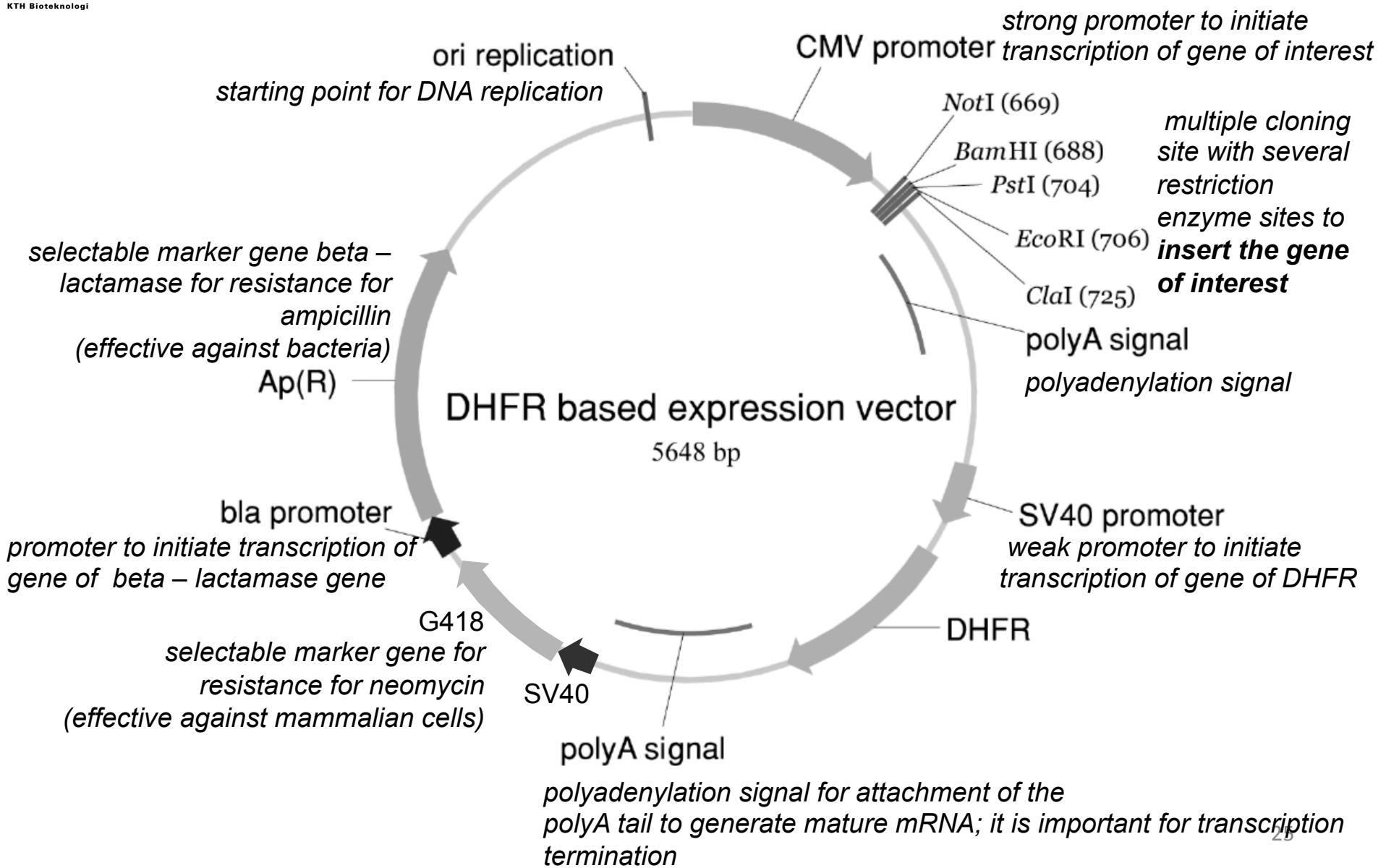
Methionine sulphoxamine (MSX) = inhibitor of GS

MSX → No cell division in absence of glutamine, for cells which are lacking GS activity

Cell expression for mammalian cell lines

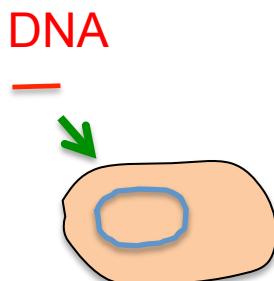
1. Multiplication of plasmid in E.coli with pressure selection by bacteria antibiotic (e.g. ampicillin)
 2. Transfection of plasmid in mammalian cells, e.g. electroporation, lipid-based lipofection
 3. Selection of surviving cells by antibiotic killing animal cells → to ensure successful gene integration, e.g. geneticin
 4. Amplification by e.g. MTX in DHFR⁻ cells
-
- One or several plasmids transfected simultaneously
 - Possibility to monitor transient expression (2-3 weeks) before stable cell line established (2-6 months)
 - Critical to obtain a stable expression

Example of vector



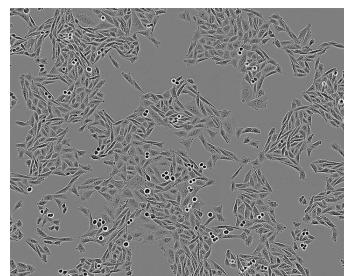
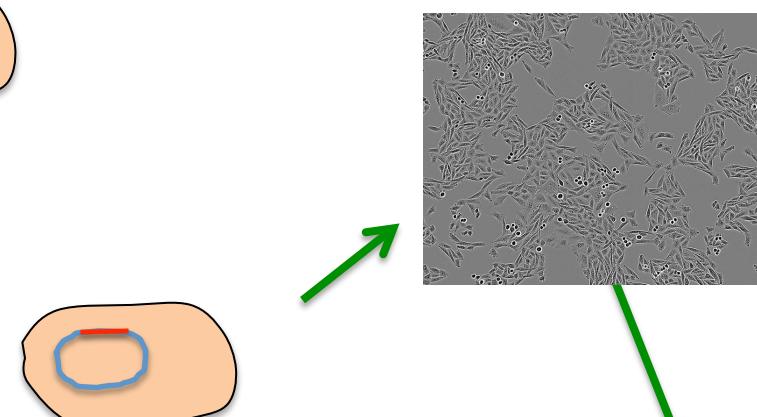
Adapted from Ng R (2009)

Development of the cell line



Generation of a new **cell line**
expressing the biopharmaceutical
→ hundreds or thousands of clones

Clone =
identical cells
issued from
one **UNIQUE**
cell



Evaluation of
expression of
biopharmaceutical, i.e.
expression level,
cell functionality,
(product quality)

Selection of a few
clones



Evaluation for process incl. stability → in
shake flasks, bioreactors

Development of the cell line (cont')

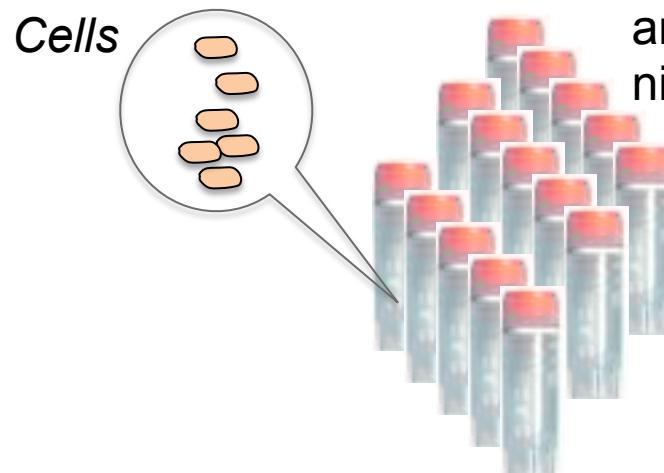


Evaluation for process incl. stability -> in shake flasks, bioreactors



Development of process for production in bioreactor

Selection of one clone



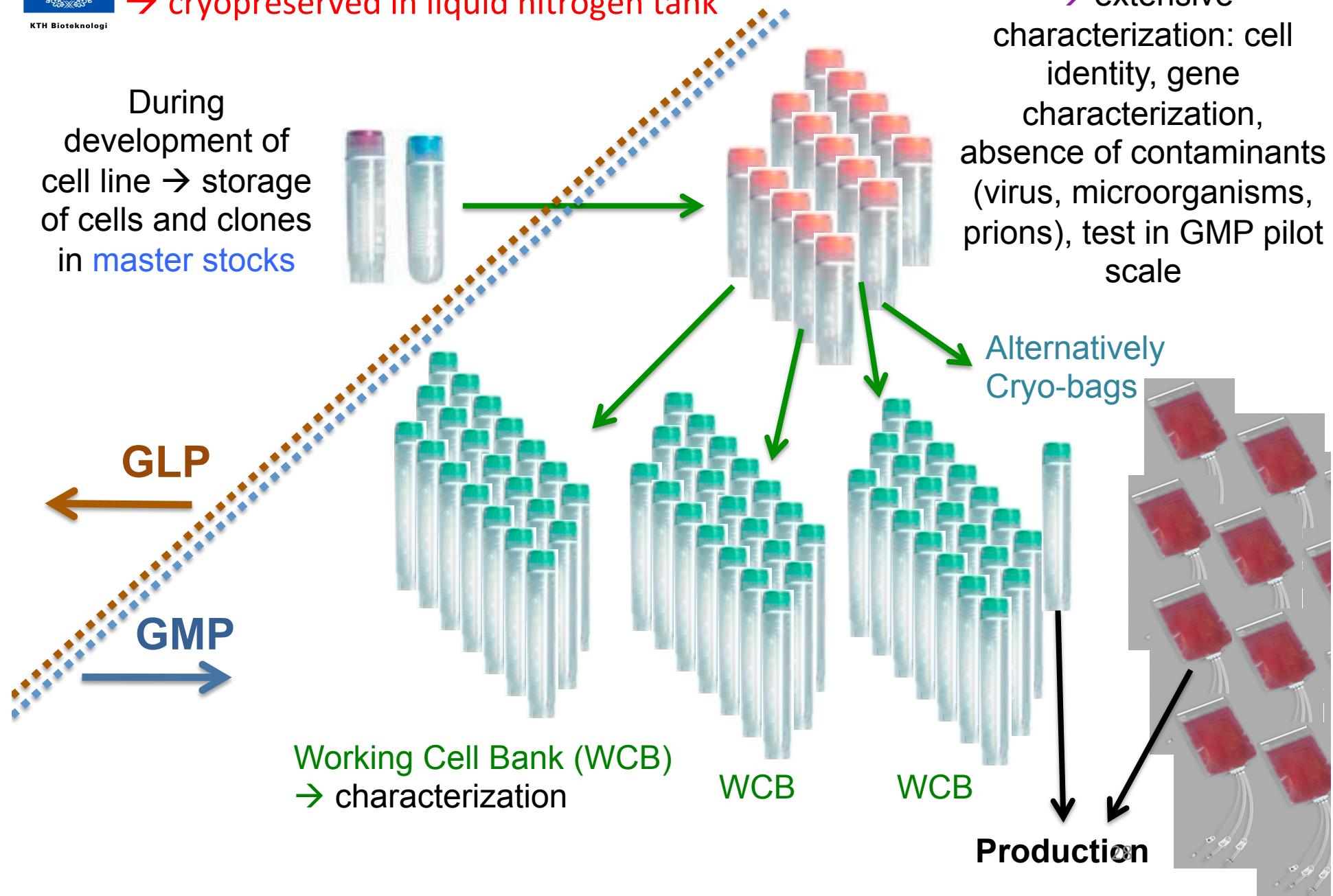
Manufacturing of Master Cell Bank and storage in nitrogen tank



Cell banking
→ cryopreserved in liquid nitrogen tank

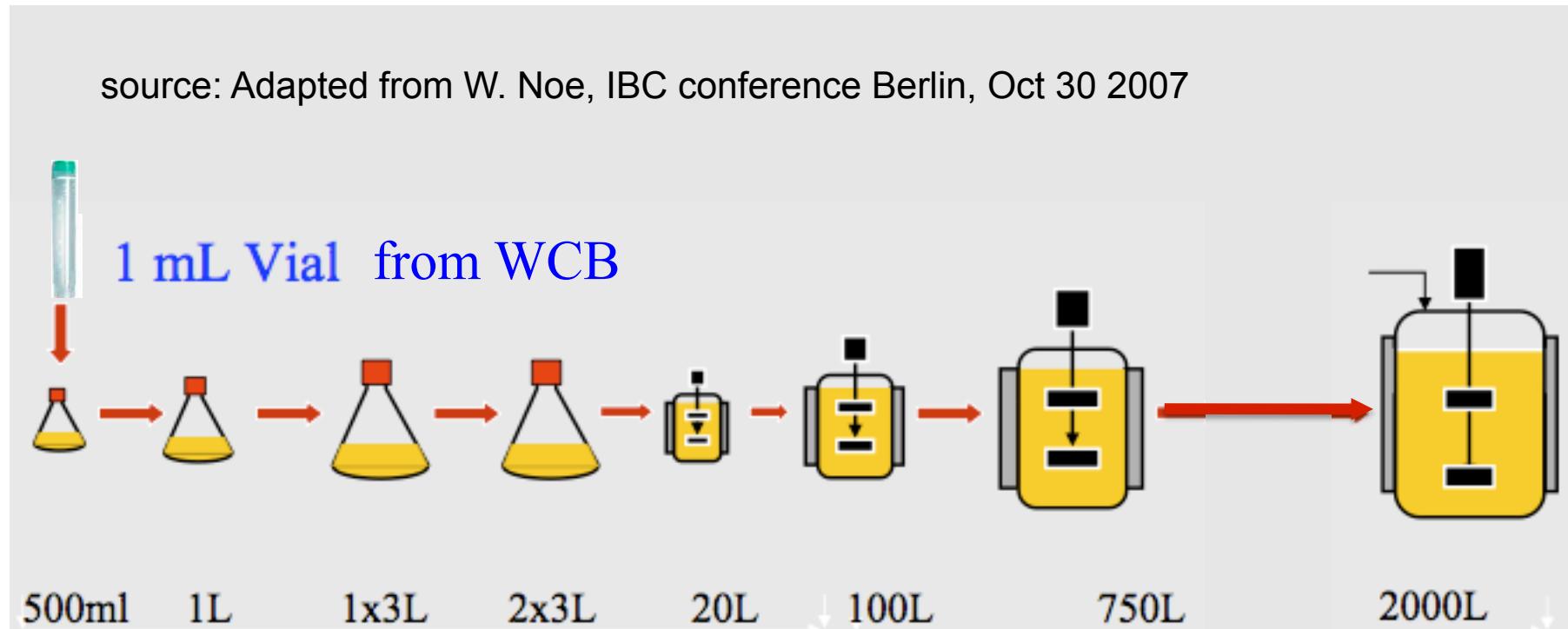
During development of cell line → storage of cells and clones in **master stocks**

Master Cell Bank (MCB)
→ extensive characterization: cell identity, gene characterization, absence of contaminants (virus, microorganisms, prions), test in GMP pilot scale



Up-stream process flow sheet for production with mammalian cell

- Cell bank (N2 tank, cryopreservation) → cell thaw from a cryo-vial
- cell expansion (e.g. shake flasks)
- cell expansion (stirred tank bioreactors ‘seed bioreactor’)
- production (stirred tank bioreactor ‘production bioreactor’)



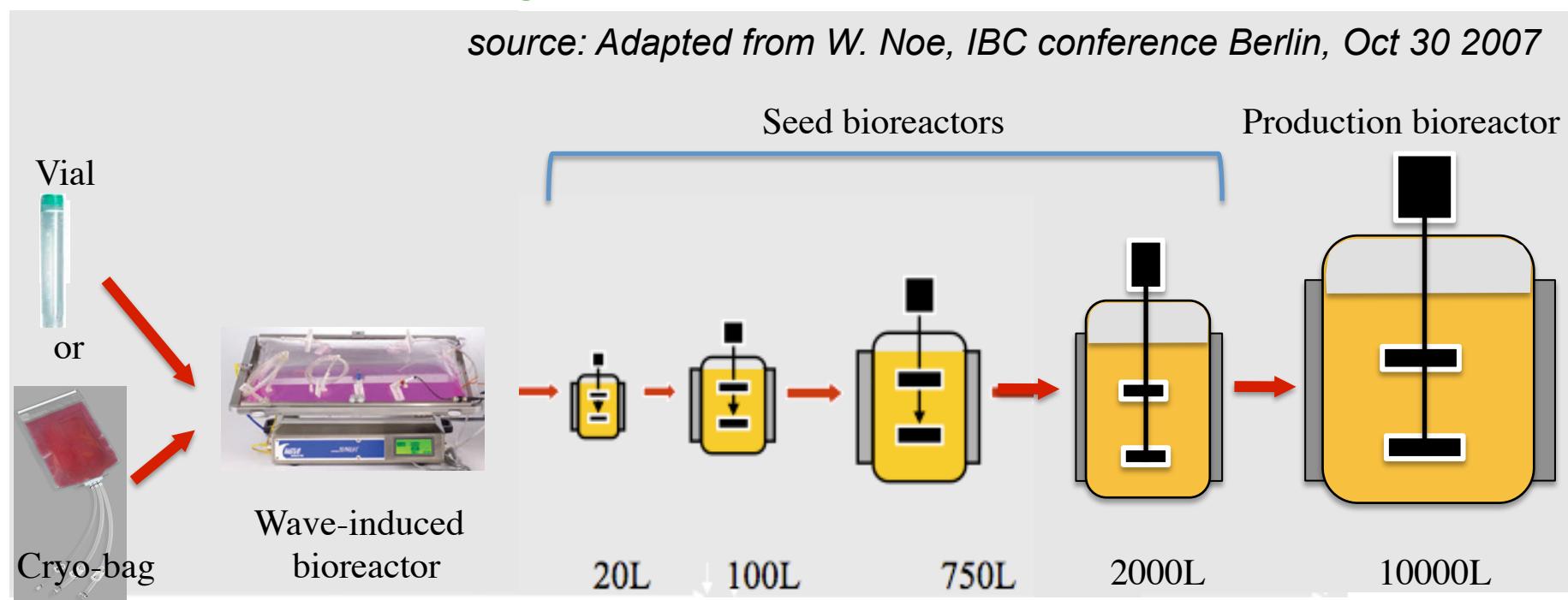
Up-stream process flow sheet for production with mammalian cell (cont')

Vial or cryo-bag

- cell expansion (e.g. increasing volumes in wave-induced bioreactor)
- cell expansion (stirred tank bioreactors ‘seed bioreactor’)
- production (stirred tank bioreactor ‘production bioreactor’)

How long time does the whole process take?

source: Adapted from W. Noe, IBC conference Berlin, Oct 30 2007

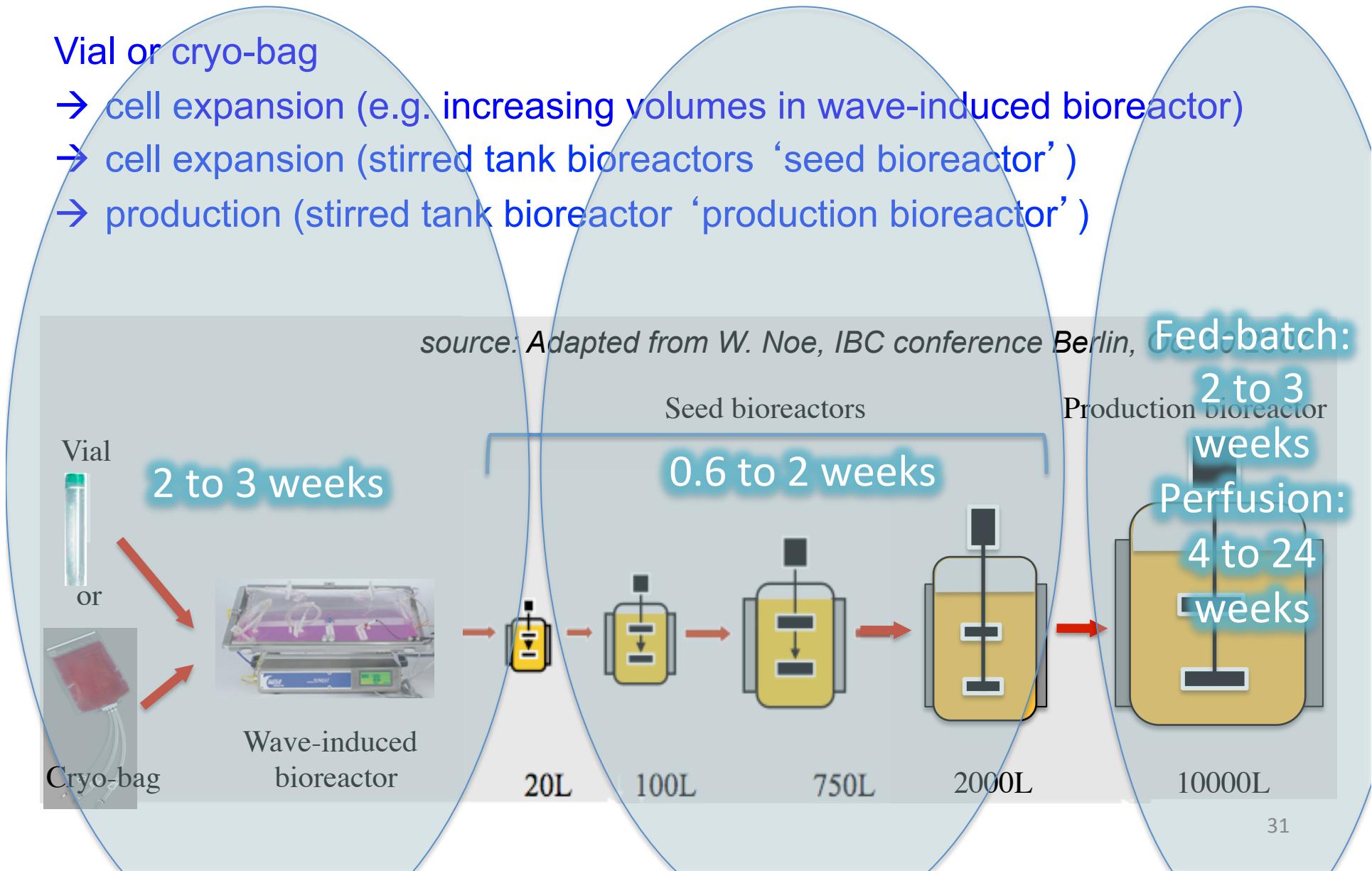


Up-stream process flow sheet for production with mammalian cell (cont')

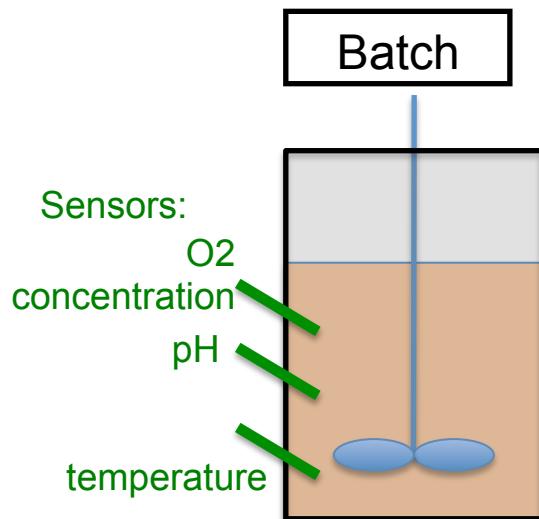
Vial or cryo-bag

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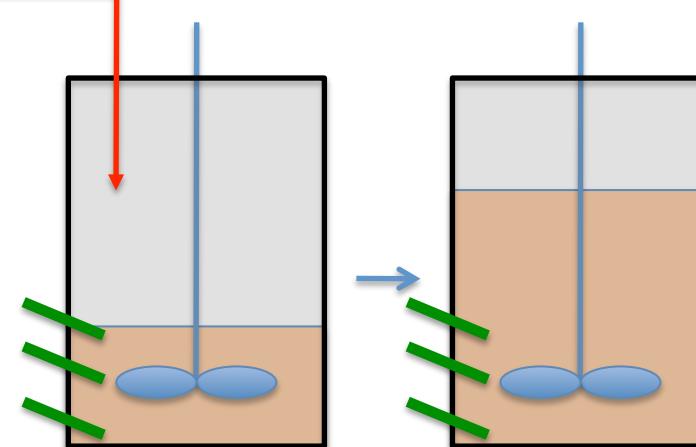
source: Adapted from W. Noe, IBC conference Berlin, 2007



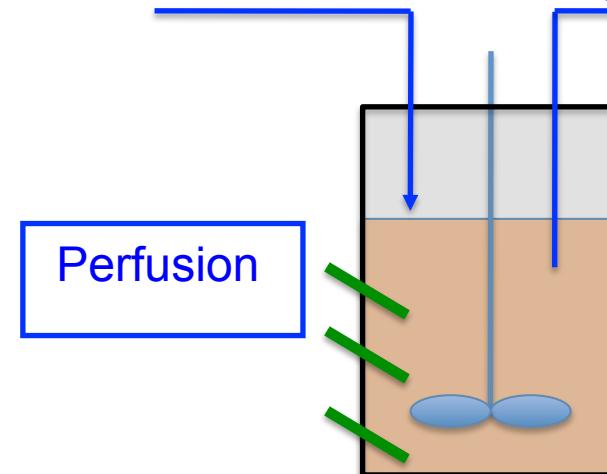
Operation: Batch, fed-batch and continuous perfusion



nutrient addition during the cultivation



continuous medium renewal during the cultivation



continuous cell broth removal and fresh medium addition during the fermentation → for microorganisms

Bioreactor

- Sampling of 15 L bioreactor (source Lonza.com)



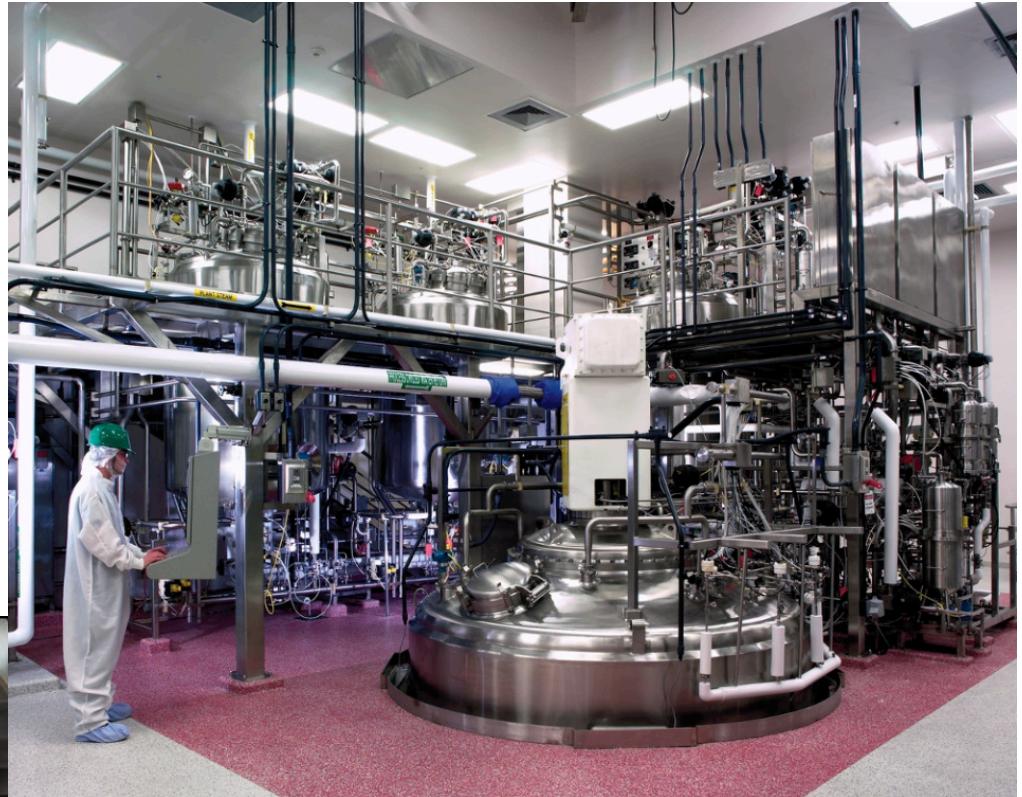
Cultivation in stainless steel bioreactors

Stainless steel production bioreactors from 500 L to 25000 L

Production bioreactors 25000 L
Genentech (Roche)



(1 of 3) Genentech lab technicians in the clean room of second Vacaville cell-culture manufacturing facility, known as CCP2, check the status of cell growth in eight large stainless-steel 25,000-liter bioreactors, at left, that will ultimately become antibody medications to treat certain types of blood cancers. (GARY QUACKENBUSH / FOR NORTH BAY BUSINESS JOURNAL) June 2017



Production bioreactors 20000 L
Lonza

Disposable equipment and new trends towards continuous processes

- Tendency to use only disposable, sterile equipment
 - reduced CAPEX (Capital Expenditure)
 - increased patient safety due to reduced risk of contamination and cross-contamination
 - higher flexibility
 - reduced cleaning validation
 - reduced labour
 - due to smaller bioreactor size, compatible with continuous processes but not with very large fed-batch processes
 - drawback = more plastic...
- Disposable equipment is used for culture and purification, e.g. disposable fermenters, disposable chromatography column for purification
- Field in expansion



Cultivation in disposable
bioreactors

Production in disposable bioreactors
up to 2000 L

source Renschler.com

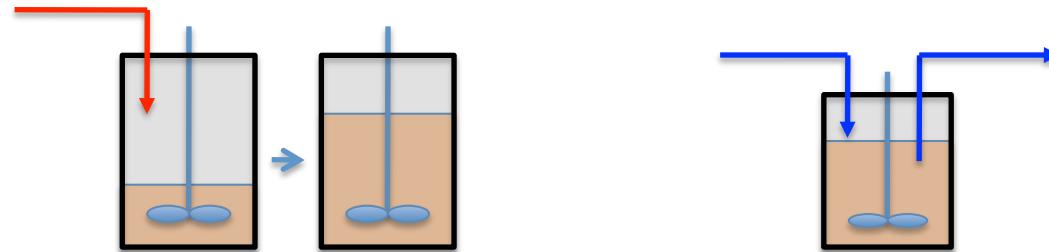


source GE.com



source Sartorius.com

Fed-batch or perfusion for mammalian cell systems?



	Fed-batch	Perfusion
Yield	high	low/high
Cell density	medium	medium → very high
Risk	low	higher (contamination)
Unstable proteins	unfavorable	ideal
Cost	comparison case by case	
Vessel size	large (up to 20 m ³)	small (up to 1-2 m ³)
Equipment		complex
Capital Investment for manufacturing	large	small(er)
Knowledge need	more biological knowledge	more technical knowledge
Disposable bioreactor	limited compatibility to sizes 1-2 m ³	good compatibility at all scales (up to 1-2 m ³)

Manufacturing of material for TOX & clinical phase

- Pilot scale
 - enough material → patients, formulation, stability study, reference material, etc.
 - reproducible
 - quantity and quality according to expectation
 - TOX → to animal
 - no need of GMP → GLP
 - better to produce with equipment same or similar to clinical material production for better understanding of process but more expensive
 - typically 1 run
 - clinical material → GMP according to batch protocol
 - takes time up front!
 - typically 1 to 3 runs

Principle for the calculation of the cultivation vessel (bioreactor)

- Annual need
 - = nr. patients x dose/patient/year
 - other factors: expiration time (24 – 36 months)
- Expected cultivation yield
- Expected purification yield
 - antibody production: 40 - 70 %
 - more complicated processes, e.g. additional PEGylation: can be 30 % or less
- Annual production
 - size of available bioreactor
 - too small or too large bioreactors → unfavorable COGS
 - availability of slot time
- Existing bioreactor sizes
 - clinical phase: 200 to 5000 L
 - commercial phase: 200 to 20000 L

Principle for the calculation of the cultivation vessel (bioreactor) through a fictive example

- Annual need
 - = nr. patients x dose/patient/year → 150000 patients @ 200 mg/year

- Expected cultivation yield → 2 g/L
- Expected purification yield → 60 %

- Annual production
 - Size of available bioreactor
 - too small or too large bioreactors → unfavorable COGS
 - availability of slot time

- Existing bioreactor sizes
 - commercial phase: 2000 L, 10000 L and 15000 L

How many runs and in which bioreactor size will you perform the production of the biopharmaceutical?

Principle for the calculation of the cultivation vessel (bioreactor) through a fictive example

- Annual need → 30000 g/year
 - = nr. patients x dose/patient/year → 150000 patients @ 200 mg/year
- Expected cultivation yield → 2 g/L
- Expected purification yield → 60 %
 - → each liter from the bioreactor will yield $2 \times 60\% = 1.2$ g/L
- Annual production to get 30000 g/year → 25000 L culture /year
 - size of available bioreactor
 - too small or too large bioreactors → unfavorable COGS
 - availability of slot time
- Existing bioreactor sizes
 - commercial phase: 2000 L, 10000 L and 15000 L → 2×15000 L / year

Principle for the calculation of the cultivation vessel for clinical phase through fictive examples

- Need for clinical study
 - = nr. patients x dose/patient/year
 - example 1 (ex 1): 50 patients @ 2 mg/treatment → 100 mg/campaign
 - example 2 (ex 2): 1000 patients @ 300 mg/treatment → 300 g/campaign
 - other: stability study, virus removal study, formulation development
 - e.g. 10 g
- Expected cultivation yield → 1 g/L
- Expected purification yield → 50 %
 - each liter from the bioreactor will yield $1 \times 50\% = 0.5 \text{ g/L}$
- Production/campaign → 11 g (ex 1) and 310 g (ex 2) /campaign
 - real need: 22 L 620 L culture
- Existing bioreactor sizes
- Other factors: **reproducibility** (ideal to perform at least 3 runs), scalability/production **representative** of larger/commercial scale
 - ex 1 → 1 x 200 L (phase 1) and 3 x 200 L (phase 3)
 - ex 2 → 2 x 1000 L (phase 1) and 3 x 5000 L (phase 3)

Cultivation process development (cont')

- Process development in shake flasks (< 1L) and bioreactors (1 – 10 L)
 - cultivation medium and feeds
 - process, e.g. fed-batch, perfusion
 - parameters e.g. pH, temperature
- Ability to be GMP, to be scaled-up and to be ‘industrial’ → pilot scale 50 L – 2000 L
- Reproducibility
- Robustness

Quality By Design: QBD

- Quality
 - “Good pharmaceutical quality represents an acceptably low risk of failing to achieve the desired clinical attributes.”
- Quality by Design (QbD)
 - “Means that product and process performance characteristics are scientifically designed to meet specific objectives, not merely empirically derived from performance of test batches.”

Janet Woodcock (2004)

source: Steven Kozlowski, M.D., Director
Office of Biotechnology Products OPS/CDER, ACPS 10/5/06

Cultivation process development

- Development of cultivation medium and feeds ≈ 50-70 components for mammalian cell
- Safety: absence of vector for virus and prion contaminations
 - strict usage (or strong tendency) for usage of components not derived from animal, in particular not from animal (incl. human) → removal of proteins animal derived or replacement by recombinant equivalent
 - serum → removal
 - proteins (e.g. transferrin, trypsin, albumin, insulin) → removal or replacement by recombinant products
 - ‘hidden’ source e.g. amino acids, detergents → replacement by alternative productions, organic synthesis, extraction from plants

→ mostly for mammalian cell systems but also for microorganism systems

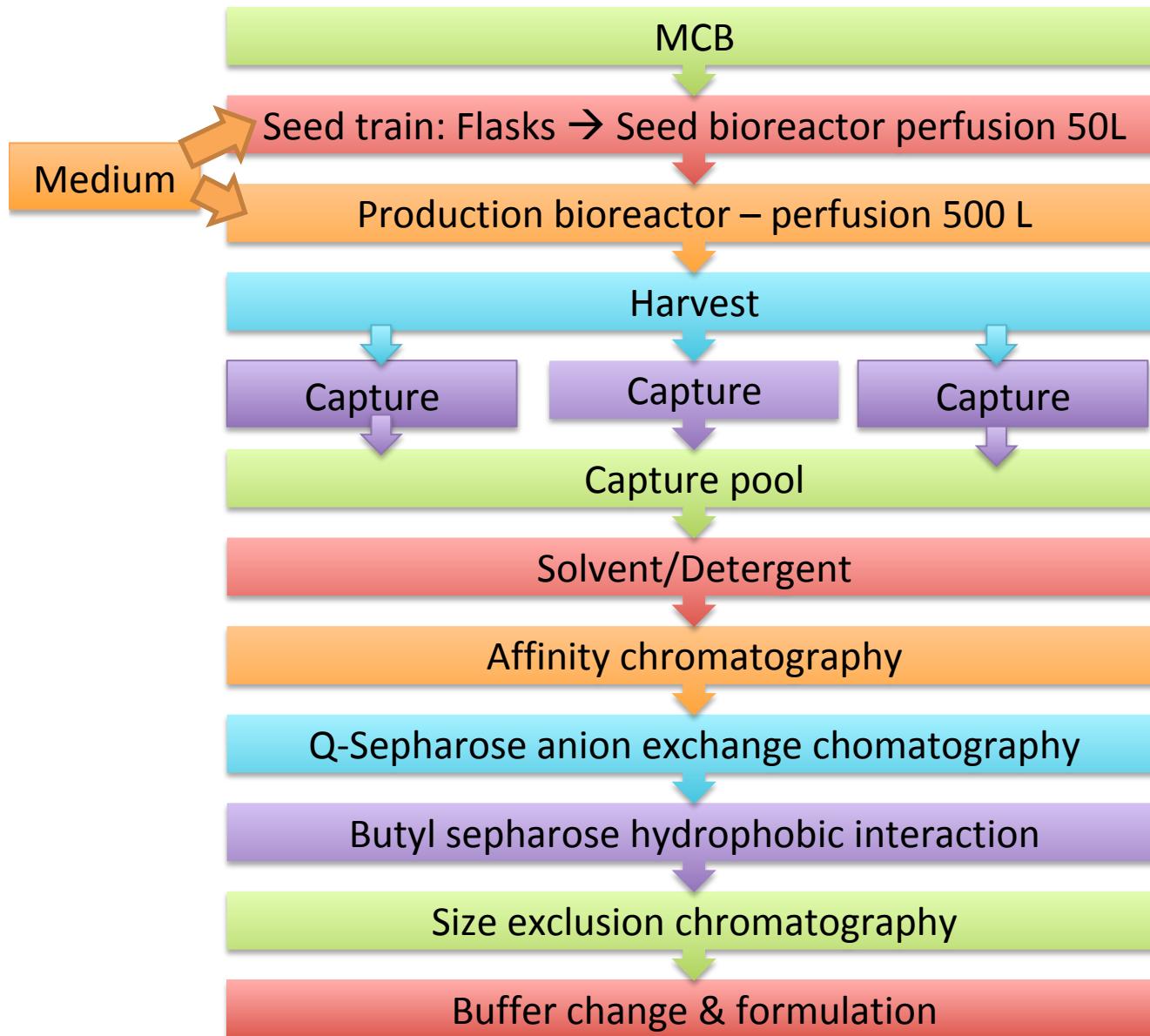
Patient safety for mammalian cell systems

- Contamination by foreign organisms
 - microorganisms -> high level of safety ensured by the process
 - » in microorganism no host/support for contaminants of humans
 - animal cells
 - » strict sterility
 - absence of microorganisms
 - absence of mycoplasma contamination
 - » risk of contamination by virus
 - known and UNknown
 - » risk of contamination by prion

→ high level of safety ensured by the process

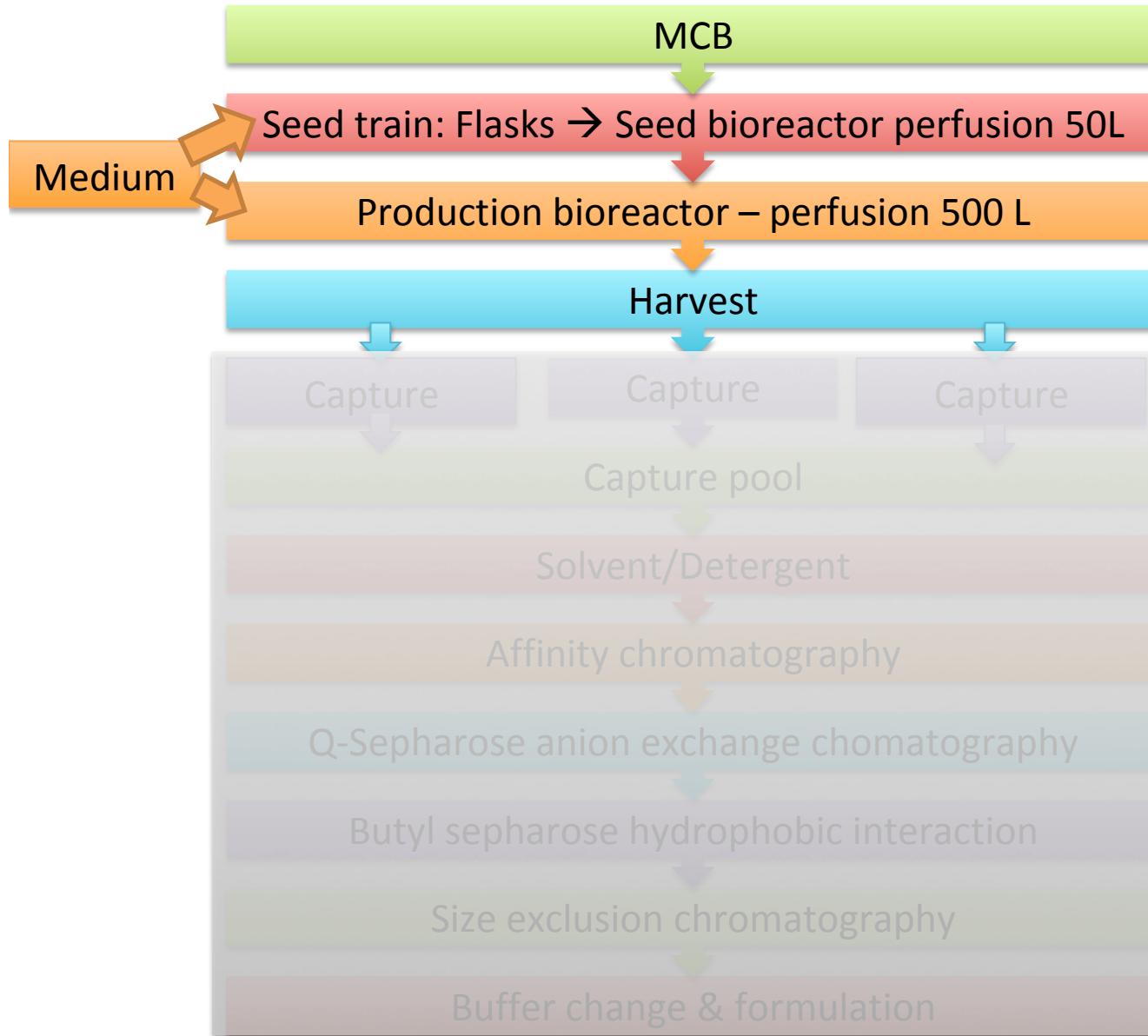


Refacto process – Recombinant factor VIII (Pfizer/SOBI)



Source: Kelley 2009

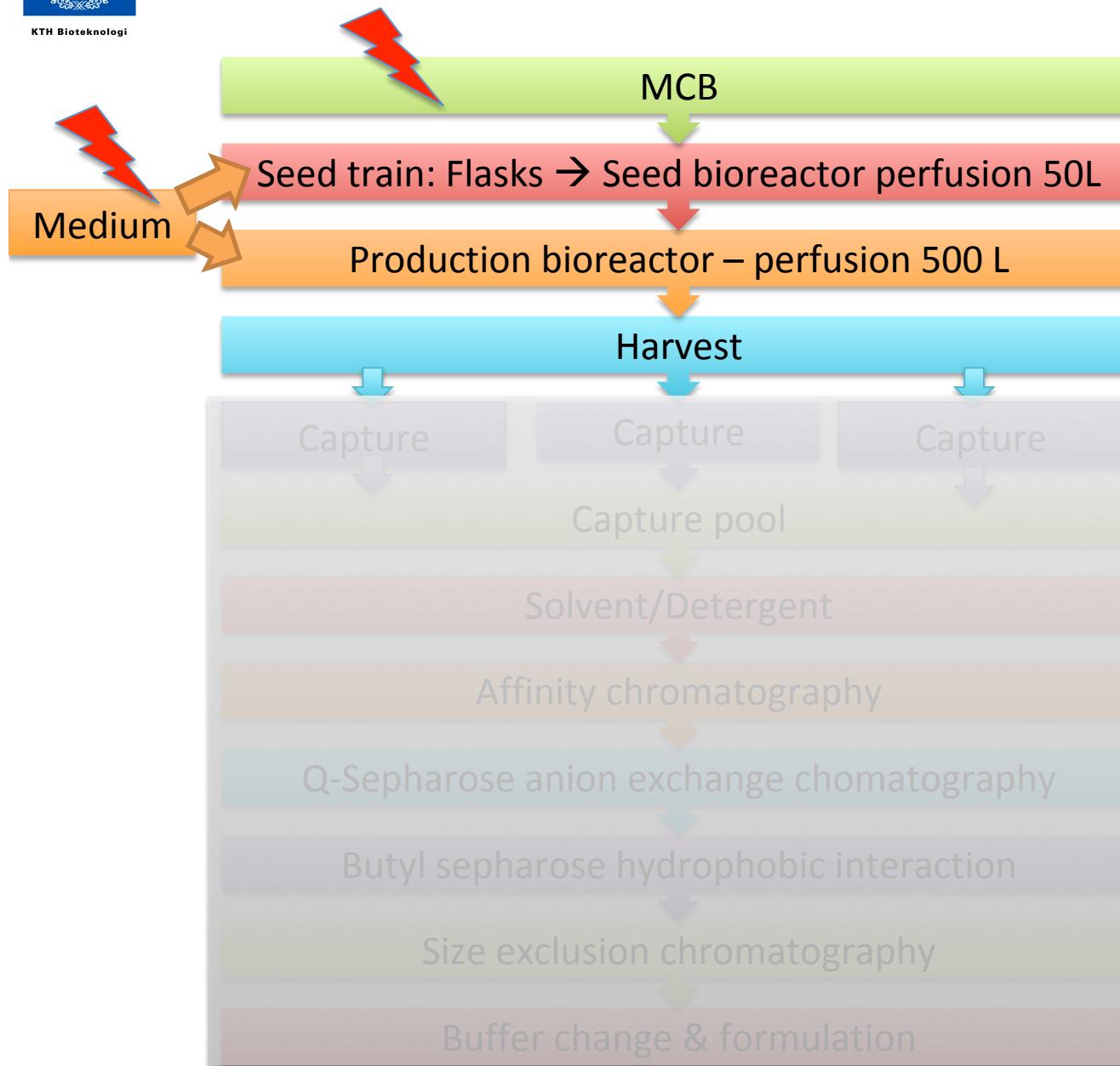
Refacto process – Recombinant factor VIII (Pfizer/SOBI)



- In which steps a virus contamination can occur?

Source: Kelley 2009

Refacto process – Recombinant factor VIII (Pfizer/SOBI)



- In which steps a virus contamination can occur?

Purification



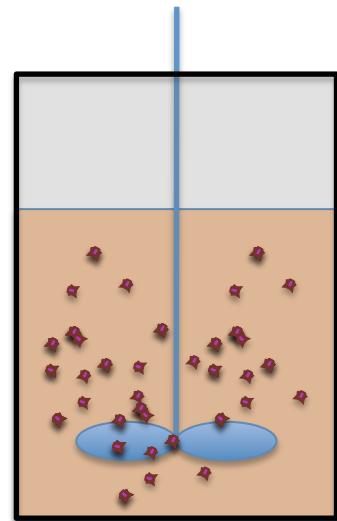
Fill Finish



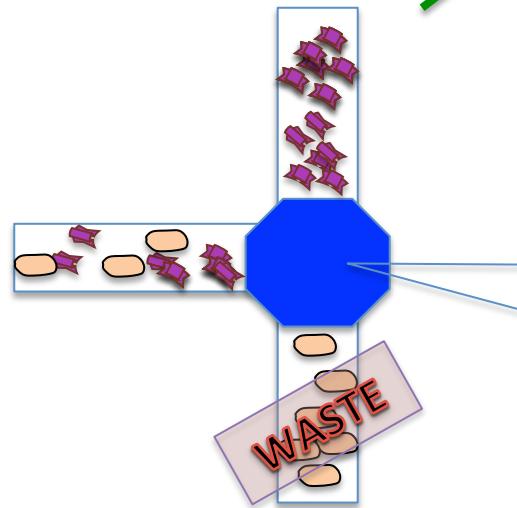
*sources: Bioengineering, GEN EngNews; GE Healthcare;
Biopharm International*

Development of purification process

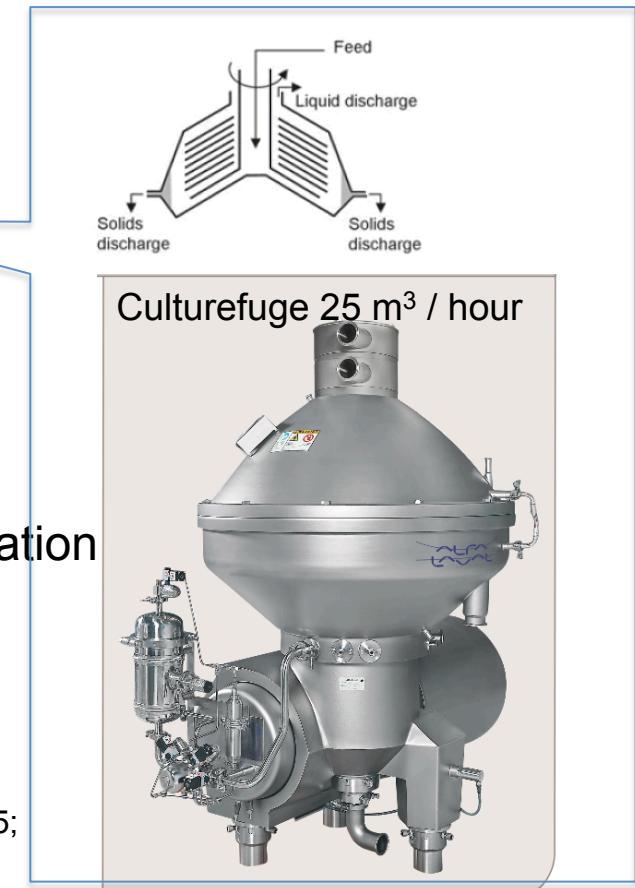
host **cell** → production of **biopharmaceutical**



Production in
bioreactor



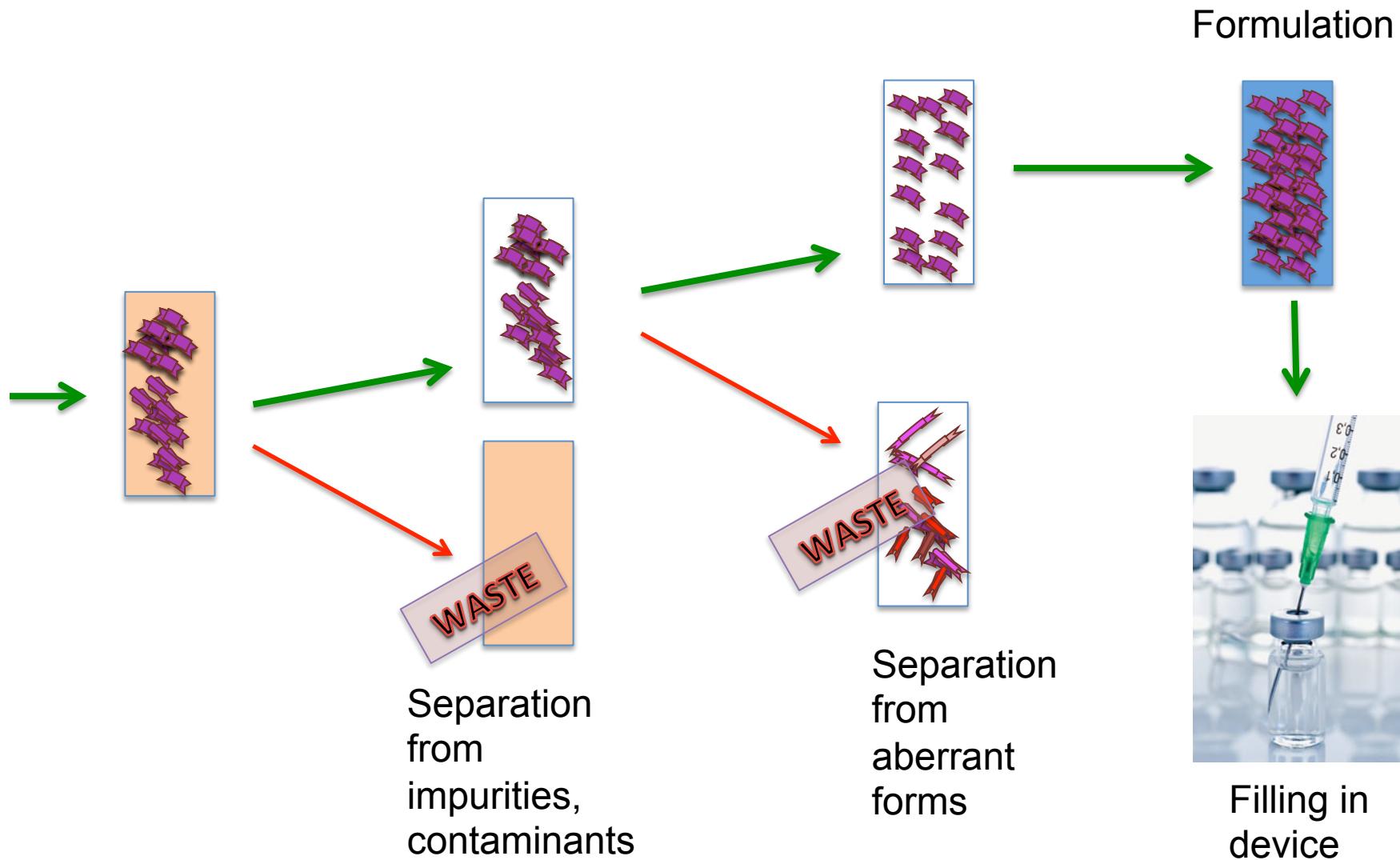
Harvest and cell separation



source Al Hattab et al 2015;
Alfalaval.com

Development of purification process

purification of biopharmaceutical



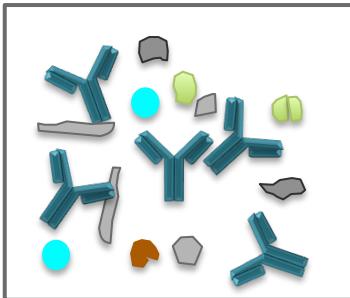
Patient safety

- Most of the biopharmaceuticals are injected
 - need purification of the product at \approx 98 or 99 %
 - impurities =
 - product related, e.g. truncated protein, aggregated protein, different glycosylation
 - process related
 - cell host: host cell protein (HCP) other than the product of interest and cell host DNA are removed by the purification process
 - equipment related, e.g. leachable from plastic, resin like protein A for antibodies
 - chemicals, e.g. antibiotics, growth factors, hormones, detergents

Development of purification process

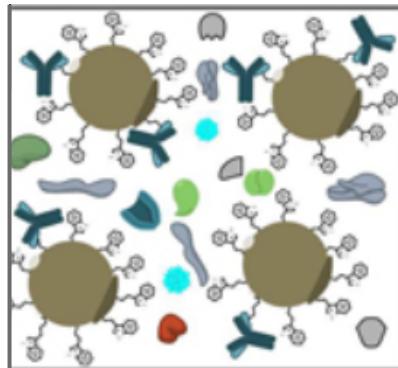
- Elimination of host related proteins, process related molecules, product related contaminants (e.g. truncation, aggregation), virus contaminants, final stable formulation
- Requires material from cultivation development
- Requires analyses for process support
- Developed in small scale in parallel with cultivation, then scaled-up
- Ability to be GMP, to be scaled-up and to be 'industrial'
- Reproducibility
- Robustness

Principle of chromatography for purification of biopharmaceuticals

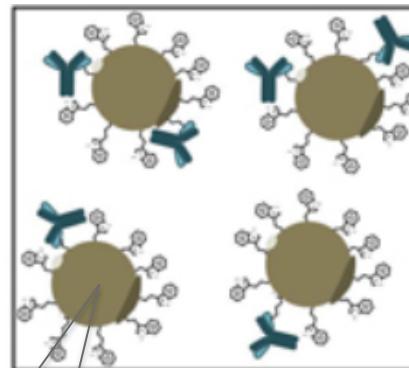


Unpurified material from culture after cell separation

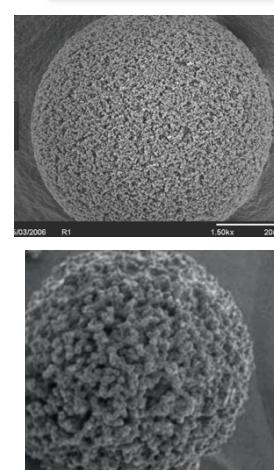
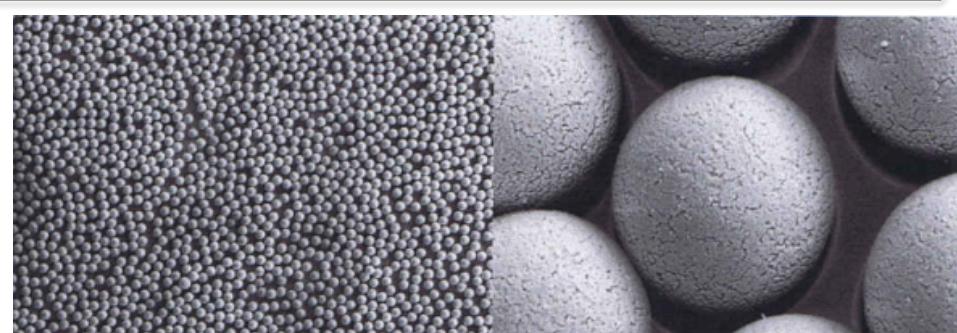
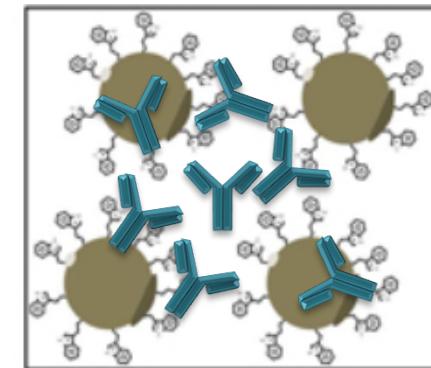
Loading and binding of antibodies to the affinity beads



Wash of impurities (i.e. non antibody molecules)



Detachment of antibodies

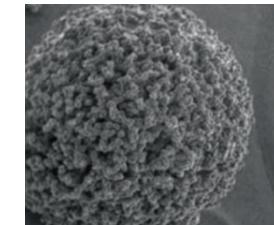
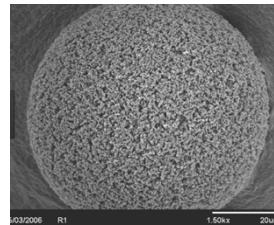
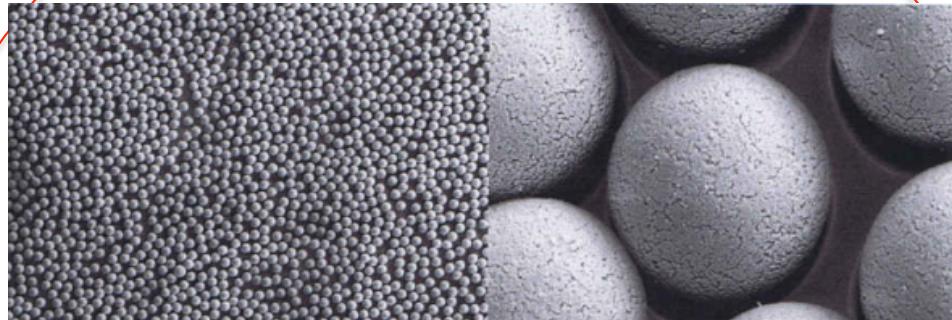


source GE.com; chromatographyshop.com; novasep.com; BioprocessIntl.com

Purified antibodies

adapted from: Maximpeptide.com

Purification process



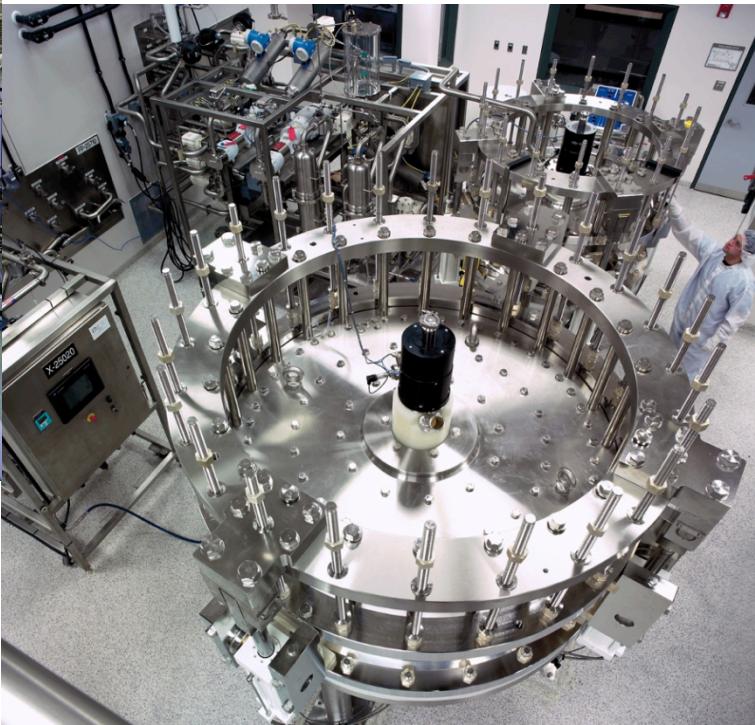
source GE.com;
chromatographyshop.com;
novasep.com; BioprocessIntl.com

Purification process



Chromatography column

Chromatography column



source GE.com; Lonza.com

Piping

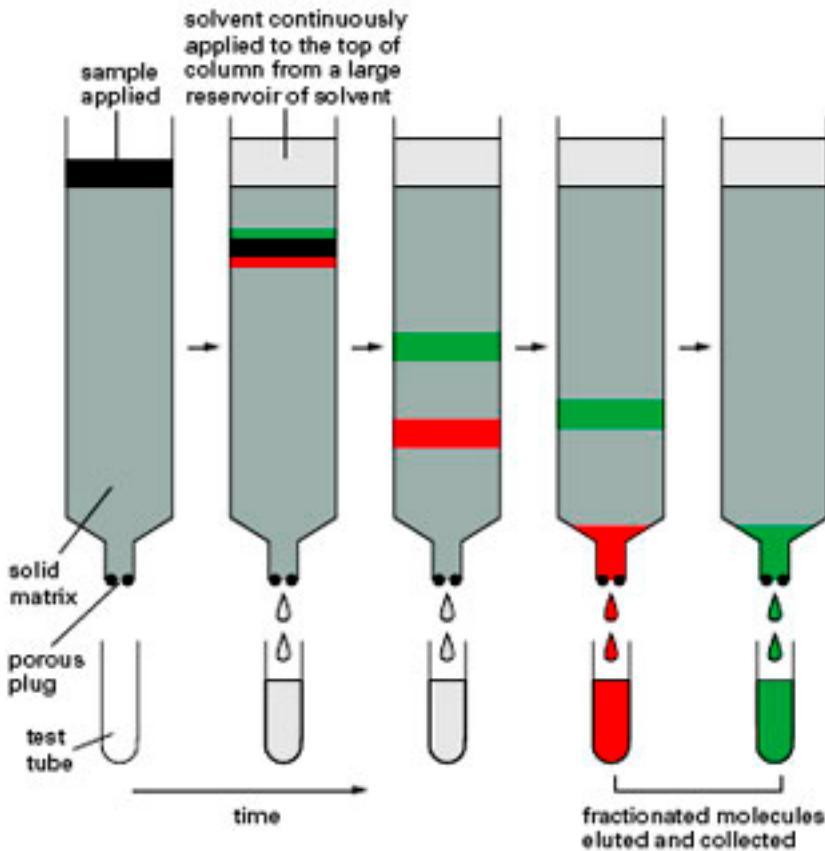


Buffer tanks

Development of purification process (cont')

Chromatography steps

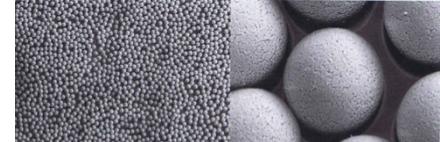
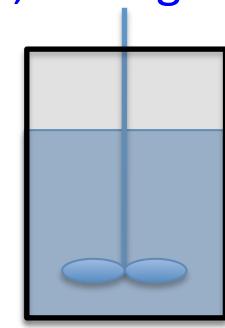
- e.g. size exclusion -> separation by protein size
- e.g. affinity chromatography -> target protein binds to a specific ligand coupled to a chromatographic matrix
- e.g. ion exchange chromatography -> protein interacts differently due to different charge distributions
- e.g. hydrophobic interaction



Reproduced from <http://www.bio.miami.edu/~cmallery/255/255tech/255techniques.htm>

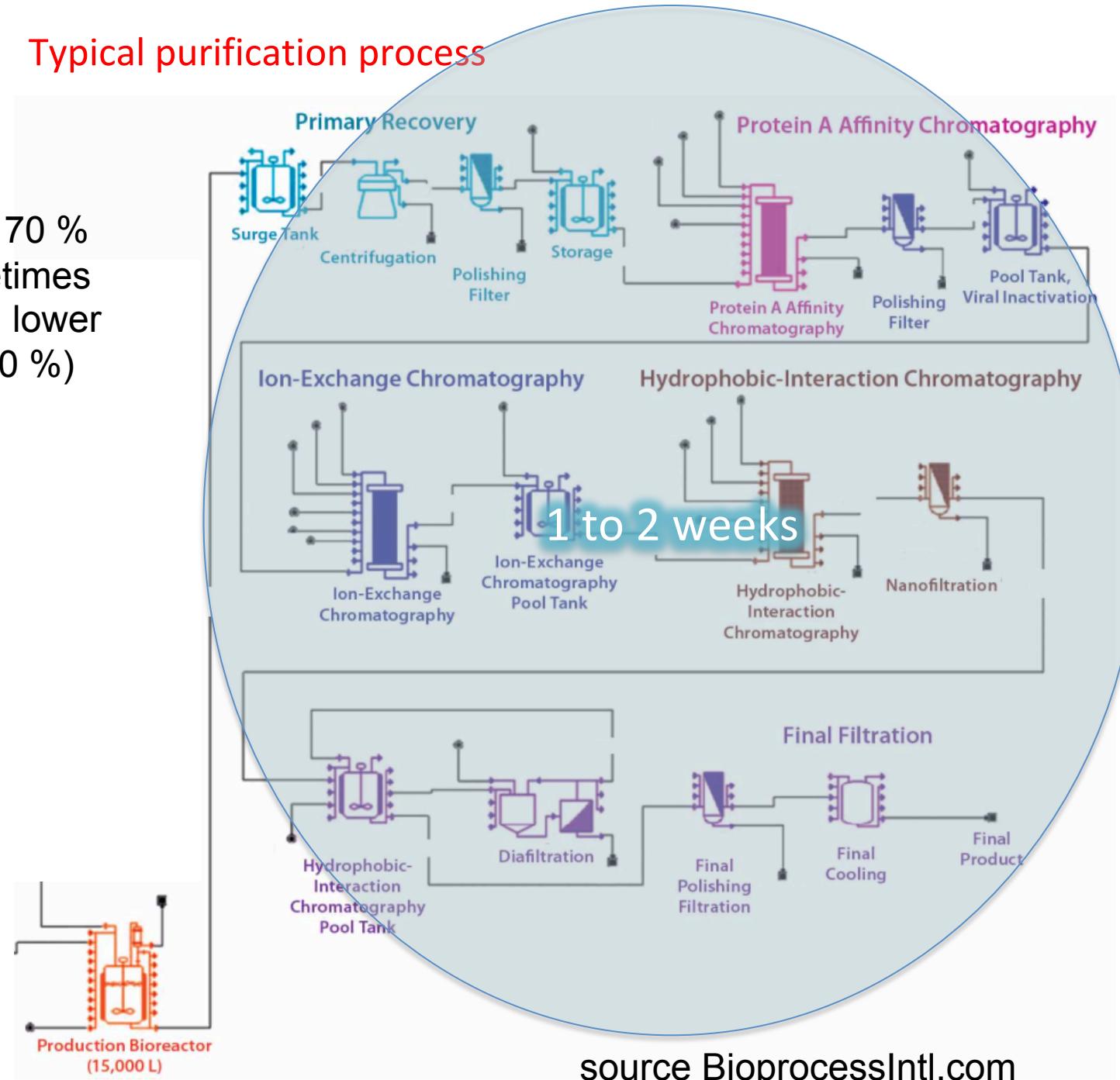
Downstream process – mammalian cell system

Process typically:

- Cell clarification (centrifugation, filtration)
 - 2 to 3 chromatography steps (one of chromatography with good virus reduction e.g. ion exchange)
 - 2 viral inactivation steps (one inactivation e.g. low pH, solvent, detergent and nanofiltration)
 - Buffer exchange steps
 - Formulation
 - biopharmaceutical substance in buffer
 - suitable for patient treatment
 - suitable for long term-preservation
- 
- 
- 
- 
- stability study → covering time of manufacturing + delivery to patients + all studies/testing's

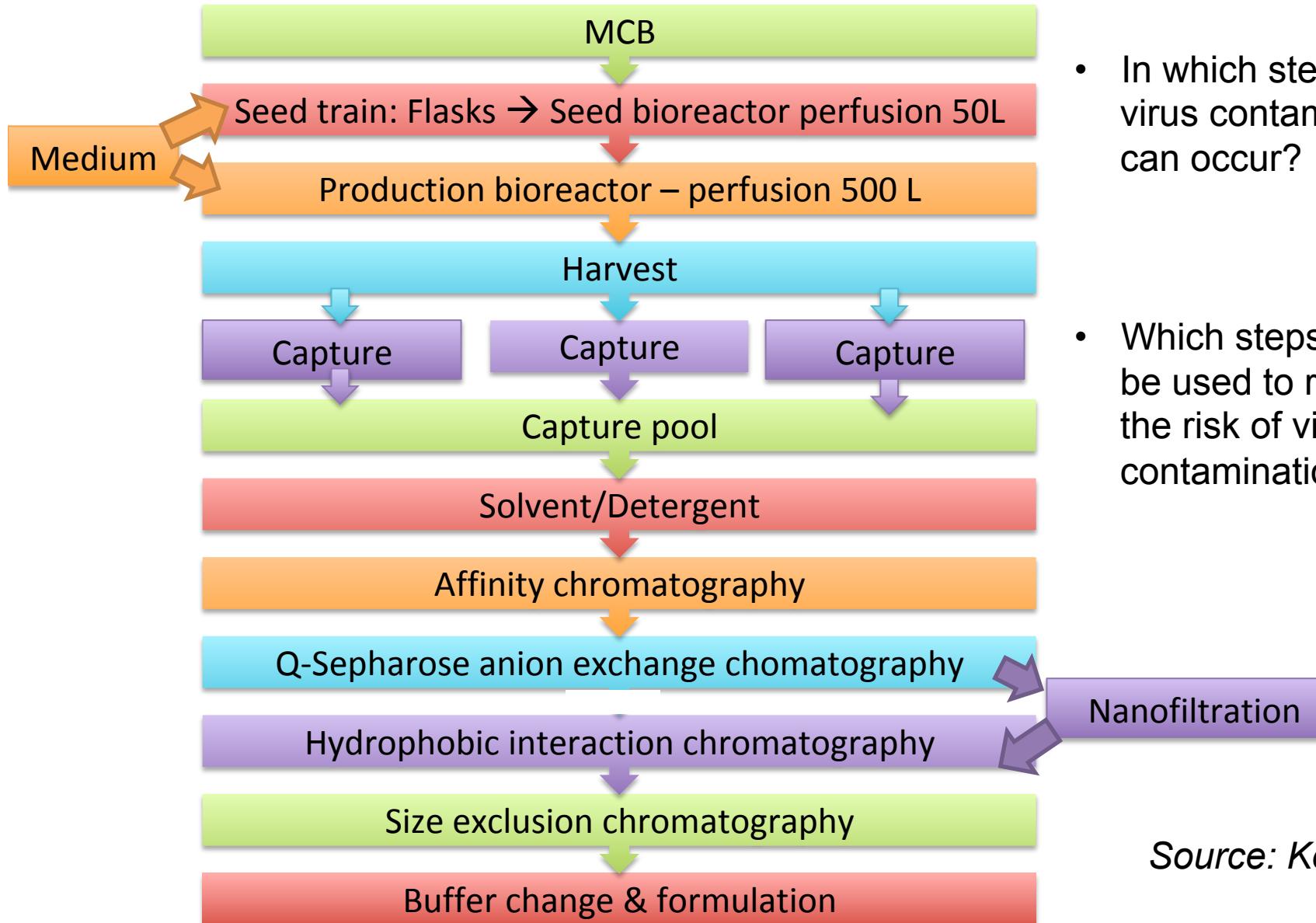
Typical purification process

Yield
 50 to 70 %
 sometimes
 much lower
 (10-20 %)



source BioprocessIntl.com

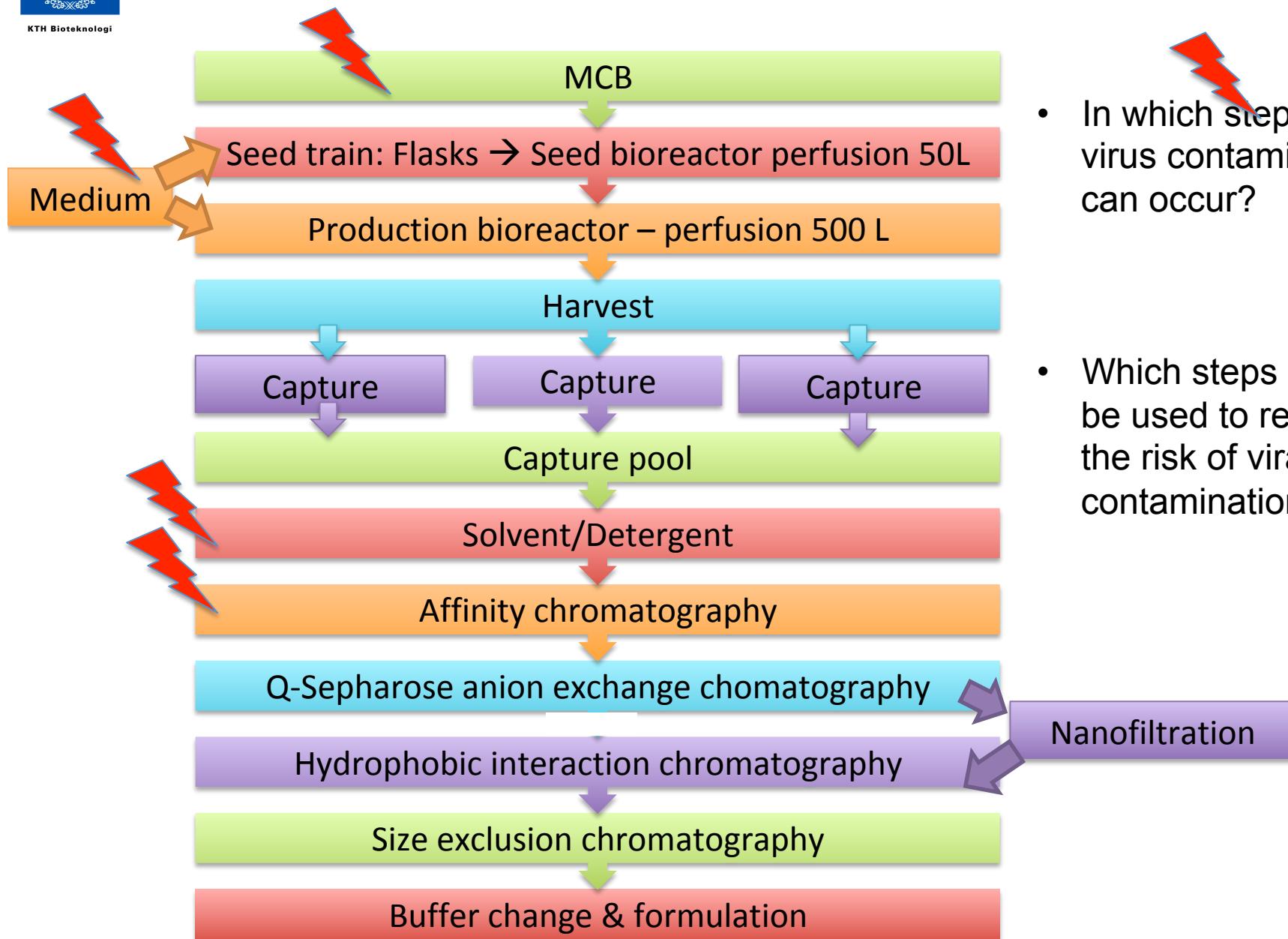
Refacto process – Recombinant factor VIII (Pfizer/SOBI)



- In which steps a virus contamination can occur?
- Which steps can be used to reduce the risk of viral contamination?

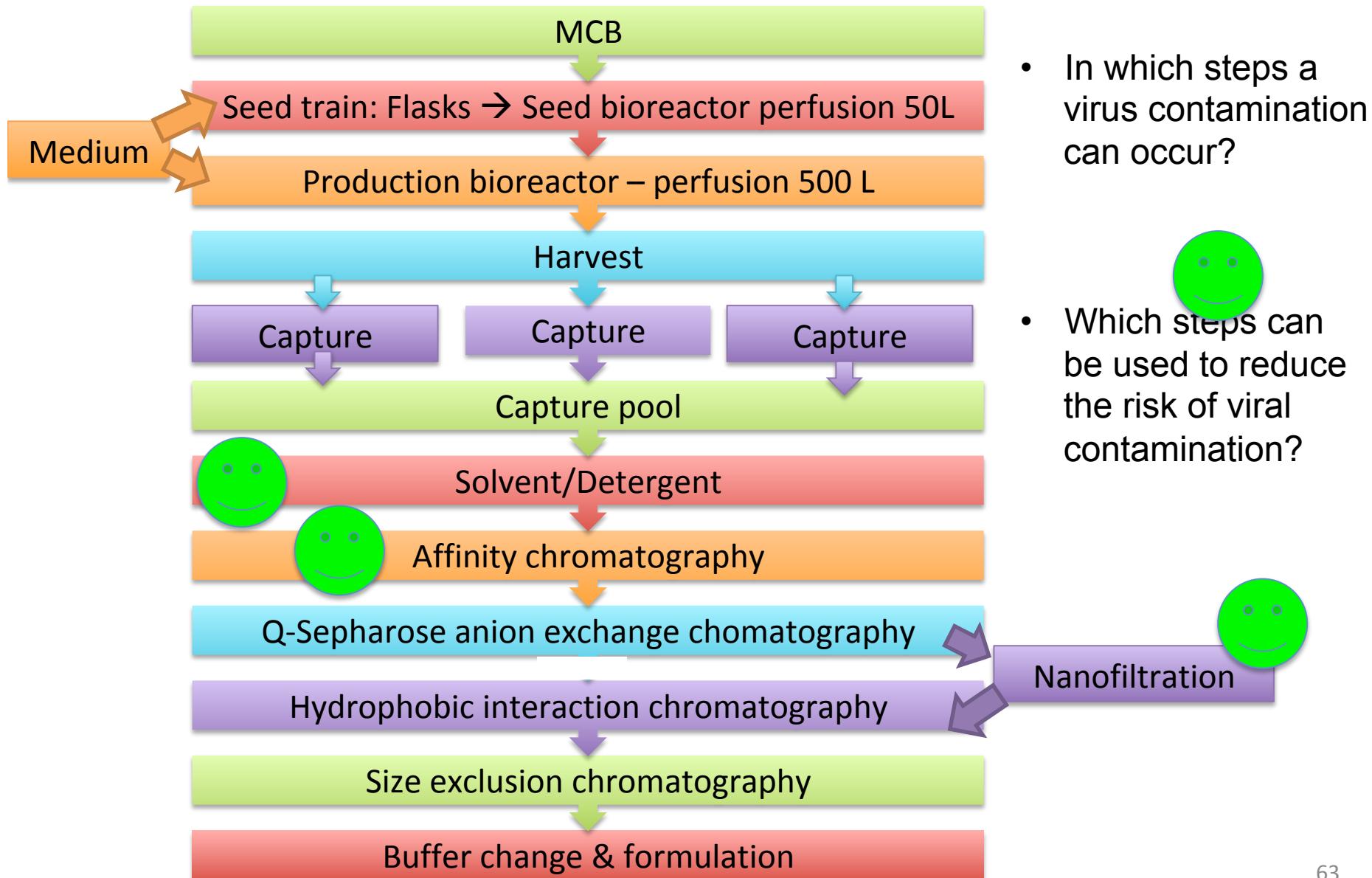
Source: Kelley 2009

Refacto process – Recombinant factor VIII (Pfizer/SOBI)



- In which steps a virus contamination can occur?
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Refacto process – Recombinant factor VIII (Pfizer/SOBI)





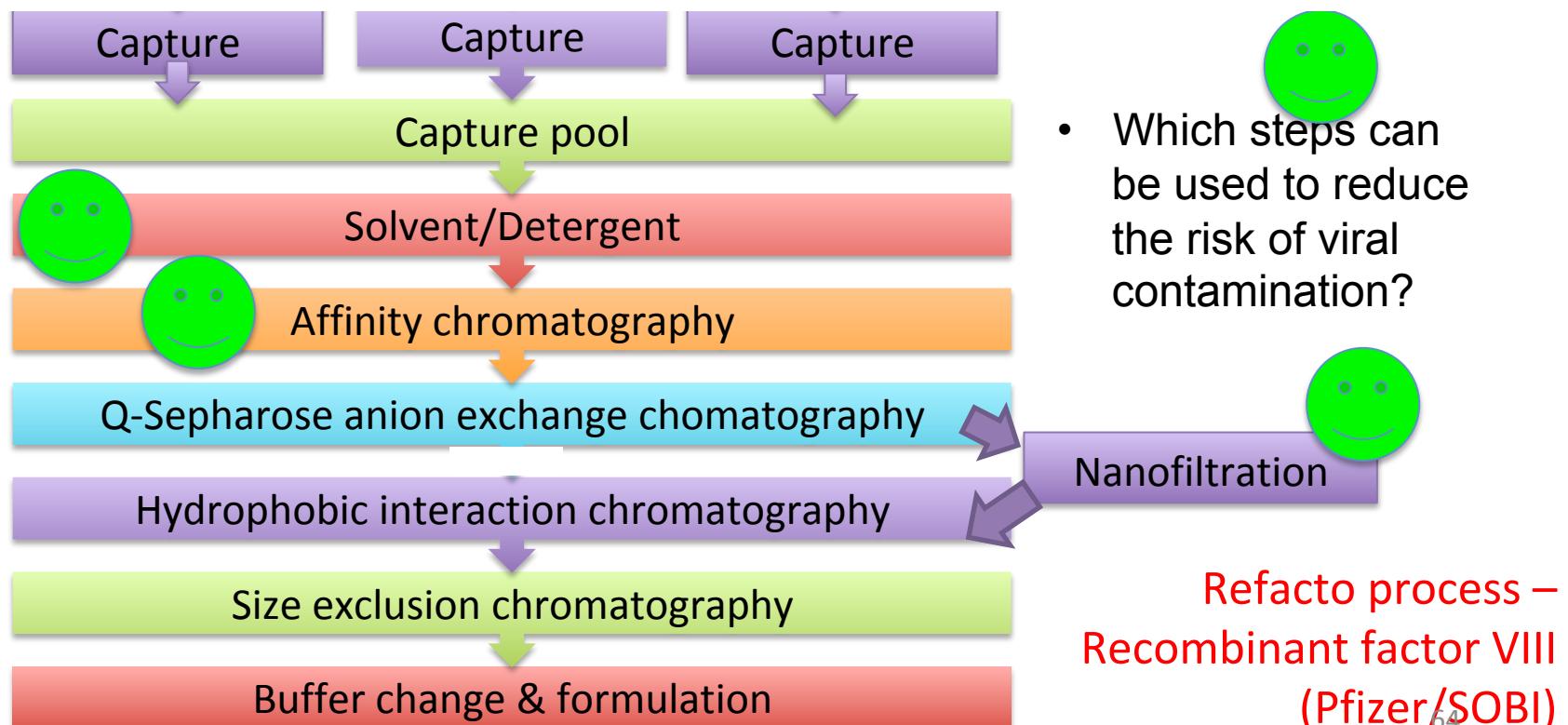
Refacto purification: viral reduction during purification

Table 2. Results of virus clearance studies (Data on File, Wyeth Pharmaceuticals, Collegeville, PA, USA).

Virus	SP Sepharose	S/D Inactivation	TN8.2 Sepharose	Q Sepharose	Planova 35N Nanofiltration	Butyl Sepharose	Total LRV*
MuLV	ND	>3.24	>2.99	ND	>5.18	ND	>11.4
MMV	1.46	ND	2.52	0.52*	0.63*	1.23	5.2
PI-3	ND	>4.93	1.51	ND	>4.95	ND	>11.4
Reo-3	ND	ND	4.40	ND	>5.93	ND	>10.3
PRV	ND	>4.90	3.13	ND	>6.00	ND	>14.0

*Log reduction values (LRV) <1.0 are not included in the calculation of total LRV.

ND, not done; Butyl, Butyl-Sepharose; env, enveloped; LRV, log removal value; MVM, minute virus of mice; nonenv, nonenveloped; PI-3, parainfluenza virus; PRV, pseudorabies virus; Reo-3, Reovirus-3; S/D, solvent/detergent; SP, SP-Sepharose; VRF, virus removal filtration; X-MuLV, xenotropic murine leukaemia virus.



Downstream process for biopharmaceutical produced from microorganisms

- Product of interest in inclusion body or leaking out (much lower yield)
- Cell harvest (centrifugation, filtration)
- Cell disruption (high pressure, bead mill) and separation of inclusion bodies (centrifugation, filtration)
- Inclusion body solubilisation (solubilizing agents, e.g., urea, guanidine HCl, or detergent Triton X-100) and re-folding (dialysis, dilution, chromatography step e.g. IEX, SEC)
- Protein purification (chromatography steps, buffer exchange steps)
- Formulation
- Less risk of virus contamination since the microorganisms are not affected or carriers of the viruses of the mammalian cells

Analyses

Analysis and protein characterisation

- Purposes → information about the product of interest for
 1. quantity (yield)
 2. activity/potency
 3. quality
 4. purity
 5. support for cultivation and purification

Which analyses can be performed to ensure these different features?
- Analysis
 - ≈ necessary information throughout development and manufacturing
- Characterization
 - deeper knowledge
 - ≈ necessary at critical stages

Analysis and protein characterisation (cont')

- Quantity (yield) e.g. ELISA, HPLC
- Activity, potency e.g. bioassay, sometimes Biacore, ELISA
- Quality and Purity
 - Identity and product related impurities (e.g. SDS-PAGE, IEF or CE-IEF, glycan map, SEC)
- Purity
 - Process related impurities (e.g. DNA (qPCR), HCP (ELISA, SDS), anti-protein A ELISA, growth factor, detergent)
- Support for cultivation and purification
- Method development takes at least 3 or 4 months → generic methods = better
- For clinical production: batch to batch robustness, structure integrity (e.g. peptide map LC-MS)
- During development: always work with a standard (!)

Release assays

Schenerman et al, 2004, BioProcess Technical
Table 2: Most frequently used lot release tests for drug substance and drug product

Method	Use, Impurities or Substances Detected	ICH Q6B Category	Quality Attribute	Drug Substance	Drug Product
Protein concentration (A_{280} absorbance)	Measure protein concentration	Quantity	Dose	Yes	Yes
High-performance size-exclusion chromatography (HP-SEC)	Aggregates, protein fragments	Purity	Size	Yes	Yes
Ion-exchange (IEC) or hydrophobic-interaction (HIC) chromatography, isoelectric focusing (IEF), or capillary IEF	Deamidation, protein fragments	Identity, purity	Charge	Yes	Yes
Capillary zone electrophoresis (CZE) or native gel electrophoresis	Deamidation, protein fragments	Identity, purity	Charge, size	Yes	Yes
Peptide mapping	Primary structure	Identity	Structure	Yes	No
Denaturing gel or capillary electrophoresis reducing or nonreducing	Protein fragments	Purity	Size	Yes	Yes
Antigen binding assay or other appropriate bioactivity assay	Potency	Potency	Activity	Yes	Yes
Host cell proteins ^a	Residual host cell proteins	Impurities	Impurities	Yes	No
DNA ^a	Residual DNA	Impurities	Impurities	Yes	No
Process-related substances and impurities ^a	Various process-related impurities	Impurities	Impurities	Yes	No
Endotoxins (<i>Limulus amoebocyte lysate</i>)	Detect endotoxins	Contaminants	Impurities	Yes	Yes
Sterility	Test for sterility	Contaminants	Impurities	Yes	Yes
pH	Measure pH	General	pH	Yes	Yes
Particulates	Impurities	Impurities	Impurities	No	Yes
Volume	Measure volume	General	Volume	No	Yes
Appearance	Evaluate color and clarity	General	Color/clarity	No	Yes

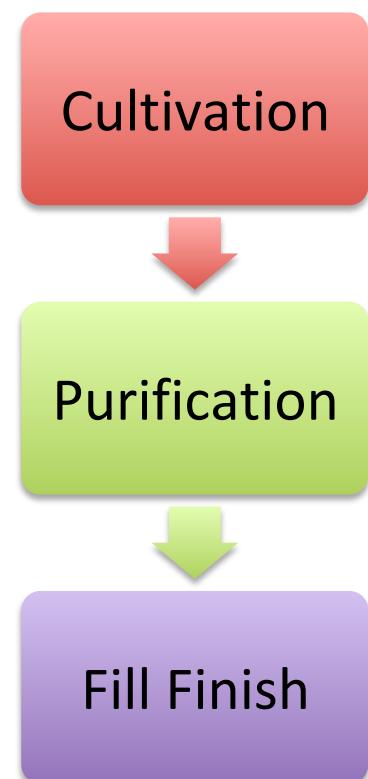
^aIt may be possible to eliminate lot-release testing for process-related substances and impurities if appropriate process clearance (removal) and process validation studies have been performed.

Specifications

Table 1. Typical release tests used for monoclonal antibody products.¹⁵ Also shown are mock specifications and data for five lots used in the clinical trials.

Test	Purpose	Specification	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
Protein concentration by A280 absorbance (mg/mL)	Quantity	50–60	55	54	55	55	56
Percent purity by high-performance size-exclusion chromatography (HP SEC)	Purity (size)	≥98.0	99.5	99.1	99.7	99.8	99.5
Ion-exchange (IEC) purity	Purity (charge)	≥95.0	98.0	97.5	98.5	100.0	98.9
Percent deamidation by percent IEC	Purity (charge)	≤5.0	2.0	2.2	1.5	1.0	1.1
Capillary zone electrophoresis (CZE)	Identity	Conforms to standard	Yes	Yes	Yes	Yes	Yes
Peptide mapping	Identity	Conforms to standard	Yes	Yes	Yes	Yes	Yes
Antigen binding assay or other appropriate	Potency	80–120%	90	95	92	101	105
Host cell proteins (ng/mg)	Impurities	≤100	10	2	5	2	5
Residual DNA (pg/mg)	Impurities	≤20	2	2	3	2	2
Endotoxin (EU/mg)	Impurities	≤0.1	0.01	0.01	0.02	0.01	0.02
pH	General	6–6.5	6.2	6.2	6.2	6.2	6.2
Volume (mL)	General	≥15	15	15	15	15	15
Appearance	General	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless

The whole process of biopharmaceutical production

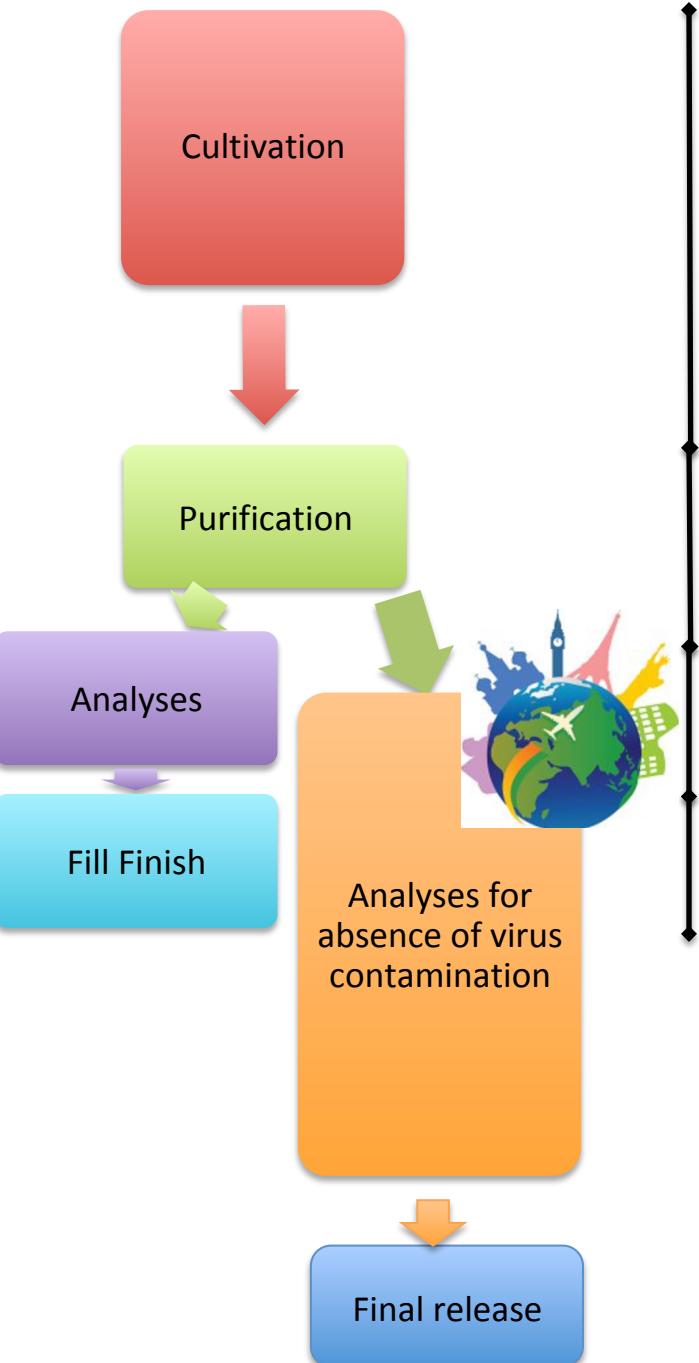


*sources: Bioengineering, GEN EngNews; GE Healthcare;
Biopharm International*

Timing for production



Often Fill Finish
at remote
location



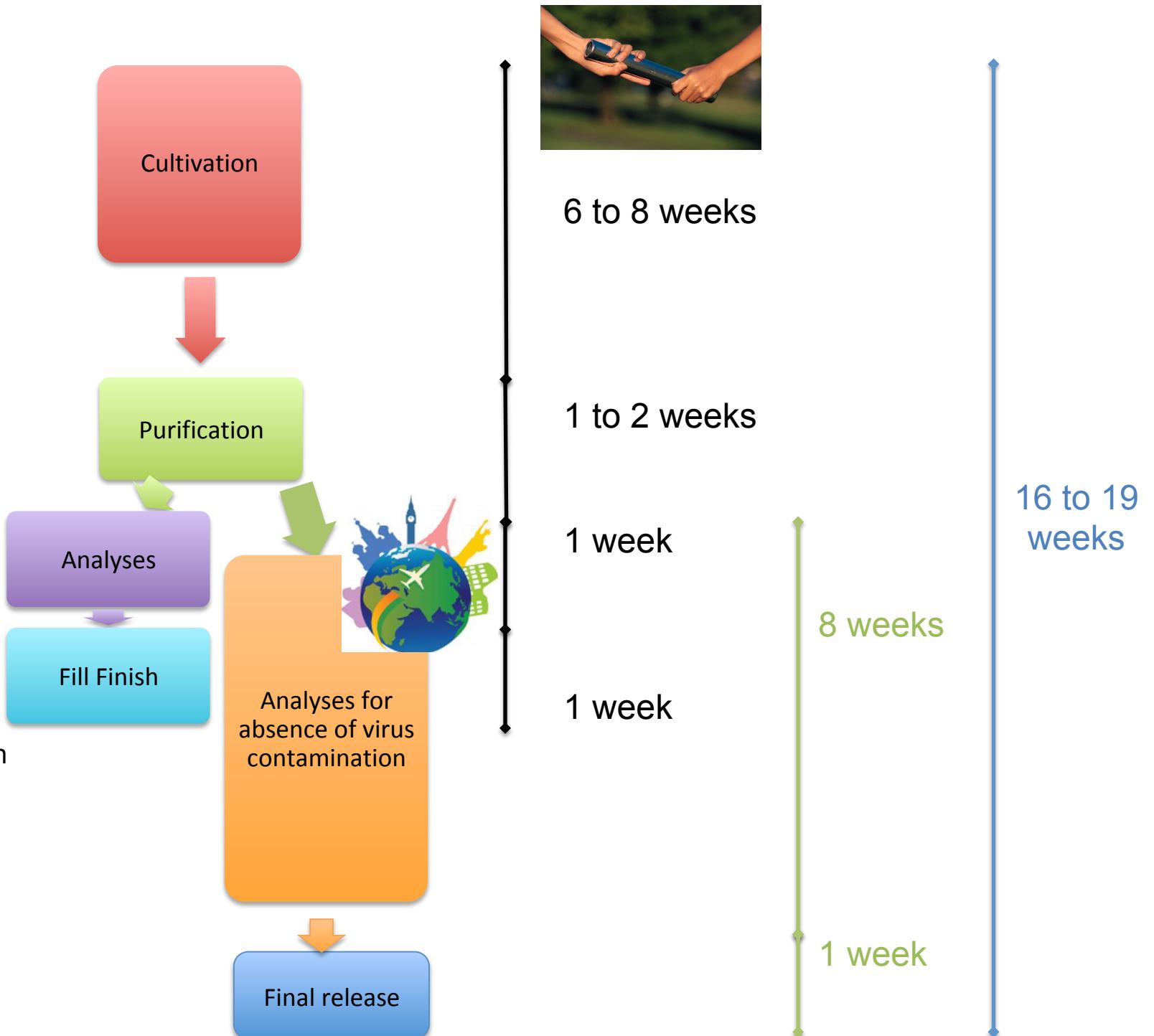
How long time takes

- the cultivation process and the purification process together
- the whole production?

Timing for production



Often Fill Finish
at remote
location



Production

- Annual need: a few grams/year to tons/year
- Product of life shelf = 2 or 3 years
- Need to produce ≈ every year
- 15 to 20 production runs per year
- Turn-over between production of 3 to 5 days (automatic)
- Production of ONE product at a time → no cross contamination
- Suites for unique production (legacy) vs. multi purpose suites (new trend)

- Examples of production

	Yield	Bioreactor size	Production / run (kg)	Production / year (kg)
Cultivation	1 to 5 g/L	5000 L	5 to 25	100 to 500
	Purification	50 to 70 %	3 to 15	60 to 300
Purification	1 to 5 g/L	20000 L	20 to 100	400 to 2000
	Cultivation	50 to 70 %	12 to 60	240 to 1200

Considerations for biopharmaceutical production and process development

Factors deciding the strategy for the production system

- The drug molecule
 - molecule size
 - factors determining the biological activity (mechanism of action): post-translational modifications e.g. glycosylation
 - factors determining the half life: glycosylation, PEGylation
 - immunogenicity profile:
 - high risk for immunogenicity by non-human systems
 - immunogenicity accepted in the case of sub-unitary vaccine where an adjuvant will be added
- Knowledge of the drug molecule and its effect in the body
 - drug mimicking existing human protein (replacement of lacking function, e.g. factor VIII coagulation)
 - antibody → known target
 - new concept

Factors deciding the strategy for the production system (cont')

- Cost from candidate to commercial product
 - large company (technology platform, equipment) or small company
 - high risk (can allow cost reduction or can imply higher cost!) or low risk (can be more expensive but allows more ‘controlled’ cost)
 - royalties for licenses (for process, drug molecule,...)
 - biogeneric (cheaper clinical trial since in principle repetition of known effect)
- Cost of Goods Sold (COGS) at commercial scale
 - annual production need: mg or kg or tons
 - price per dose
 - number of patients

Considerations for commercial biopharmaceutical production

- Correct quality of the protein
 - biological activity / function
 - half time in blood circulation
 - purity
 - patient safety
- Compliance
 - legal duty with purpose of patient safety
 - control of the biopharmaceutical quality is ensured
 - » by analyses according to Specifications but also
 - » by control of production process
- Enough quantity to cover demand
 - production (yield, product quality)
 - logistics (time for production, expiring time)
 - COGS (Cost of Goods Sold)



Considerations for biopharmaceutical process development

- Correct quality of the protein
 - biological activity / function → **often not known**
 - half time in blood circulation → **often not known**
 - purity → **contaminants not known**
→ patient safety
- Compliance
 - legal duty with purpose of patient safety
 - control of the biopharmaceutical quality is ensured
 - by analyses according to Specifications → **have to be decided**
 - by control of production process → **ROBUSTNESS, scalability, no change of cell line**
- Enough quantity to cover clinical phase(s) study **AND** stability study, formulation development, virus clearance, reference production
 - production (yield, product quality)
 - logistics (time for production, expiring time) → **product stability not known**
 - COGS of commercial process in mind

✓ completely



Considerations for biopharmaceutical process development

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✓ completely



AS FAST AS POSSIBLE

AS CHEAP AS POSSIBLE

